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Functional morphology of gustatory centers in the brain of the channel catfish, *Ictalurus punctatus*

Lamb, Charles Franklin, IV, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1991

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FUNCTIONAL MORPHOLOGY OF GUSTATORY CENTERS IN THE BRAIN OF THE CHANNEL CATFISH, ICTALURUS PUNCTATUS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Zoology and Physiology

by
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B.A., Humboldt State University, 1983
M.S., Louisiana State University, 1986
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On a more personal note, I thank my parents, Charles and Barbara Lamb, for their persistent admonishments on the permanence of education and the pursuance of elusive goals, and my wife, Judy Lamb, for her support and patience throughout the process. It is to these people that this dissertation is dedicated, not as a culminating triumph but as a struggling first step.

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Abstract

Gustatory nuclei in the isthmic region of the metencephalon and in the posterior diencephalon of the channel catfish, Ictalurus punctatus, were examined anatomically and electrophysiologically.

Neurons in the superior secondary gustatory nucleus (nGS) responded to taste and tactile stimulation of large peripheral receptive fields (RFs) that often included the epithelia of the oropharyngeal cavity and whole extraoral body surface. Taste responses to amino acids and nucleotides were dose-dependent, with detected thresholds from micromolar to millimolar concentrations. The RF organization within the nGS did not display the somatotopy present in the medullary gustatory nuclei. Anatomical labeling of secondary gustatory fibers showed that the distribution of fibers from different portions of both the facial (FL) and vagal (VL) lobes of the medulla overlapped within the nGS.

Efferents from the nGS ascend in the tertiary gustatory tract to the caudal inferior lobe, where they terminate caudally in the nucleus lobobulbaris (nLB) and nucleus centralis (nCLI), and rostrolaterally in the nucleus diffusus (nDLI). Secondary projections from the FL also terminate in the nLB and in the nucleus subglomerulosus (nSG). The nLB forms three cell groups (caudal - nLB, rostrolateral - rl nLB, parvicellular - nLBp) which project to the FL, VL, and telencephalon, respectively. Cells from the nCLI project throughout the caudal inferior lobe and to the acousticolateral torus semicircularis and

telencephalon, while the nDLI and nSG have intrinsic connections within the inferior lobe. The lateral thalamic nucleus (nLT) projects from this region back to the nGS.

Taste responses similar to those from nGS units were recorded from units in the nCLI, nLB, rl nLB, nLBp, and nLT, supporting the proposed gustatory role for these nuclei. Tactile responsiveness was different between nuclei in the caudal inferior lobe. Units from the nCLI and nLB had lower spontaneous activity than those from other nuclei and typically had RFs including the whole ipsilateral extraoral body surface. Units from the rl nLB and nLBp had RFs that often included both oral and extraoral surfaces, bilaterally, but rl nLB RFs typically included the whole body while nLBp RFs were often restricted to the head or mouth.

Chapter I

Introduction

The sense of taste in fishes has been an active field of research for more than one hundred and fifty years. Weber first described taste buds on the pharyngeal palatal organ of carp in 1827 and, in 1851, Leydig identified similar terminal (taste) buds in the epithelial layer of the extraoral skin of fishes (cited by Landacre, '07). Anatomical studies tracing the peripheral branches of the cranial and spinal nerves in teleosts found that the taste buds within the oropharyngeal cavity were associated with branches of the glossopharyngeal (IX) and vagal (X) cranial nerves, while the extraoral terminal buds were associated with branches of the facial (VII) nerve (Herrick, 1899, '01). The termination of these cranial nerves in the medulla was identified as the fasciculus communis (Kingsbury, 1897; Herrick, 1899, '06; Johnston, '02) following Strong's (1895) description of the communis (taste) system of cranial nerves in amphibians. Furthermore, Herrick (1899) suggested that the fasciculus communis of teleosts was homologous to the fasciculus solitarius of mammals and that the communis system as described for teleosts was functionally similar to the mammalian special visceral sensory (taste) system.

Early behavioral studies provided support for the gustatory nature of the communis system of teleosts. Numerous studies with catfish found that feeding behavior was elicited when the barbels, fins, or flanks, regions with large numbers of terminal buds (Herrick, '03), were stimulated with food or food extracts (Herrick, '03, '04; Parker, '08, '10; Parker and Sheldon, '12; Olmsted, '18). Those studies, along with a later study of feeding behavior in the gourami

(Scharrer et al., '47), concluded that the taste system was responsible for feeding responses in teleosts and, further, that the responses to chemical stimulation were augmented by concurrent tactile stimulation. In addition, Hoagland ('33), using the then recently developed electrophysiological techniques, recorded both taste and tactile nerve responses from facial (VII) nerve fibers that innervate the maxillary barbel of catfish. This initial investigation of the taste responses of the peripheral facial fibers of catfish led to the recent characterization of the exquisite responsiveness of the facial (Caprio, '75, '78; Davenport and Caprio, '82), as well as glossopharyngeal and vagal (Kanwal and Caprio, '83) fibers in the channel catfish to stimulation of the extraoral and oropharyngeal surfaces, respectively, with amino acids (see Caprio, '84, '88).

The extensive development of the communis system of many teleosts, particularly of the ostariophysine silurids and cyprinids, led many early investigators to study the correlated brain structures of these fishes. Mayser, in 1881, first described the enlarged vagal and facial ("lobus trigemini") lobes of the medulla of the carp, as well as a highly developed fiber tract ("secondary vago-trigeminal tract") from these lobes to a large nucleus ("Rindenknoten") in the isthmic region, ventral to the cerebellum (cited by Kingsbury, 1897, and Herrick, '05). Kingsbury (1897), in an anatomical study of the medulla of seventeen species of fishes, correctly associated these structures with the communis system, since they were more developed in species with greater numbers of taste buds. Kingsbury also identified

the peripheral input to Mayser's lobus trigemini as the facial (VII) and not the trigeminal (V) nerve as proposed by Mayser. Kingsbury's description of the communis system of teleosts was confirmed and expanded by Herrick ('01, '05, '06, '07, '44), who studied the termination of the facial, glossopharyngeal, and vagal nerves in the fasciculus communis, as well as the efferents of the medullary gustatory nuclei. Herrick correctly renamed the lobus trigemini, the secondary vago-trigeminal tract, and the Rindenknoten of Mayser as the facial lobe, ascending secondary gustatory tract, and superior secondary gustatory nucleus, respectively (Herrick, '05). Herrick also described the tertiary gustatory tract ascending from the secondary gustatory nucleus to the posterior inferior lobe, although he was unable to identify the specific termination sites of these tertiary fibers (Herrick, '05). Herrick's descriptions of the gustatory pathways in teleosts were later confirmed in a comparative study of the connections of the medullary facial nuclei of various vertebrate groups (Barnard, '36), and in a behavioral and neuroanatomical study of the gourami (Scharrer et al., '47). It is interesting to note that the corresponding ascending projections from the fasciculus solitarius of mammals were only recently identified (Norgren and Leonard, '73; Norgren, '78), even though they were suggested by Herrick as early as the turn of the century (Herrick, 1899) and accurately described by him in catfish and carp in 1905.

With the recent development of modern neuroanatomical techniques, numerous studies of the gustatory system in silurids (Finger, '76, '78, '83; Finger and Morita, '85; Morita and Finger,

'85; Kanwal and Caprio, '87) and cyprinids (Luiten, '75; Morita et al., '80, '83; Kiyohara et al., '85; Puzdrowski, '87; Finger, '88a) have further described the afferent and efferent connections of the medullary gustatory nuclei. An interesting result from these studies is that the termination patterns of peripheral afferents into both the facial (Finger, '76; Kiyohara et al., '85; Puzdrowski, '87) and vagal (Kanwal and Caprio, '87; Finger, '88a) lobes are organized as topographical representations of the extraoral and oropharyngeal epithelial surfaces, respectively. In addition, electrophysiological studies have confirmed this organization through the identification of topographically organized receptive fields of neurons in both the facial (Peterson, '72; Marui and Funakoshi, '79; Marui and Caprio, '82; Marui et al., '88; Hayama and Caprio, '89) and vagal lobes (Kanwal and Caprio, '88) of these teleosts. Both anatomical (Finger, '78; Morita et al., '80, '83) and electrophysiological (Marui, '81) results have indicated that the superior secondary gustatory nucleus might also be topographically organized, although less distinctly than the representations in the medullary nuclei.

The present study is an anatomical and electrophysiological investigation of the gustatory centers associated with the ascending gustatory pathways from the facial and vagal lobes in the channel catfish, Ictalurus punctatus. The first section is concerned with the organization of gustatory information within the superior secondary gustatory nucleus. This section involves a description of the responsiveness (to taste and tactile stimulation) of units obtained

from the superior secondary gustatory nucleus and the correlation between those responses and the efferents of the facial and vagal lobes that terminate in the secondary gustatory nucleus. In an attempt to identify anatomical substrates for potential mechanisms underlying the processing of gustatory information in teleosts, the second section identifies the specific terminations of tertiary gustatory fibers in the caudal diencephalon and the connections of the recipient nuclei of those fibers. To further characterize gustatory information at this level and to identify differences in responsiveness between different levels of the gustatory system, the final section is an electrophysiological investigation of the individual nuclei in the caudal diencephalon. All three sections represent crucial steps toward understanding the gustatory system in catfish and toward learning basic principles of the neural organization of the taste system as it is exemplified by different vertebrates.

Chapter II

Convergence of Oral and Extraoral Information in the Superior Secondary Gustatory Nucleus of the Channel Catfish

INTRODUCTION

Gustatory information is transmitted to the brain of vertebrates by the facial (VII), glossopharyngeal (IX), and vagal (X) cranial nerves. In many teleosts, the gustatory system is highly developed, corresponding to the importance of gustation for food localization and feeding behavior (Herrick, '04; Bardach et al., '67; Atema, '71; Johnsen and Teeter, '80). This development is evidenced by the peripheral distribution of taste buds on the extraoral epithelium and within the oropharyngeal cavity in both silurid (Herrick, '01; Atema, '71) and cyprinid (Kiyohara et al., '85) fishes. Further, these fishes are characterized centrally by prominent enlargements of the visceral sensory column of the medulla associated with their gustatory development (Herrick, '06; Evans, '31; Evans, '52; Luiten, '75; Finger, '78; Morita et al., '80). In cyprinids, the primary afferents of the gustatory system terminate in the facial (FL), glossopharyngeal, and vagal (VL) lobes, respectively (Barnard, '36; Morita et al., '80). In silurids, however, only the FL and VL are present (Herrick, '06), and the glossopharyngeal afferents terminate in the intermediate nucleus of the facial lobe (nIF) and in the vagal lobe (Kanwal and Caprio, '87). The termination of gustatory afferents in both FL (Finger, '76; Marui and Caprio, '82; Kiyohara et al., '85; von Bartheld and Meyer, '85; Puzdrowski, '87; Kottrschal and Whitear, '88; Hayama and Caprio, '89, '90) and VL (Kanwal and Caprio, '88) is topographically arranged within each nucleus according to peripheral receptive fields (RFs). Most of the ascending secondary neurons from

the medullary nuclei join the secondary gustatory tract (2G) to terminate in the superior secondary gustatory nucleus of Herrick (nGS), located in the isthmic region ventral to the cerebellum (Herrick, '05; Shanklin, '35; Barnard, '36; Finger, '78; Morita et al., '80, '83). This pathway is thought to correspond in amphibians to the projection from the nucleus of the fasciculus solitarius to the superior visceral-gustatory nucleus (Herrick, '17, '44), and in mammals to the projection of the secondary gustatory neurons from the nucleus of the solitary tract (nST) to the parabrachial nucleus (PBn) of the pons (Norgren, '78).

The facial and glossopharyngeal/vagal taste systems in ictalurid catfish are anatomically distinct within the medulla (Finger and Morita, '85; Morita and Finger, '85) and are responsible for different behavioral patterns. The FL is important for food searching behavior and for food selection, while the VL mediates swallowing (Atema, '71). Further, anatomical studies indicate that ascending secondary efferents from the facial and vagal lobes maintain a structural separation within the nGS of catfish (Finger, '78), goldfish (Finger, '83), and carp (Morita et al., '80, '83). Electrophysiological results from the nGS in the carp indicate that RFs from the anterior extraoral epithelium project to the ventral region of the nucleus (Marui, '81), but the individual RFs of nGS cells are larger, and the somatotopy less distinct, than those recorded from cells in the FL (Marui, '77; Marui and Funakoshi, '79). No electrophysiological evidence has been reported relating the receptive fields of the facial

and glossopharyngeal/vagal systems within the nGS of teleosts. In this study, the anatomy of the nGS in the channel catfish, Ictalurus punctatus, is described, and projections from the VL and FL to the nGS are examined. In addition, neurons within the nGS are examined electrophysiologically to determine the relative size and organization of their receptive fields to both mechanical and chemical stimulation.

MATERIALS and METHODS

Electrophysiology -

Twenty-eight channel catfish, weighing from 30 to 125 g, were immobilized with intramuscular injection of Flaxedil (gallamine triethiodide, 0.5 mg/kg) and secured in a Plexiglass container. The gills and oral cavity were perfused with aerated, charcoal-filtered city tap water (artesian well water), and supplemental doses of Flaxedil were administered as required. The dorsal surface of the head was anaesthetized by topical application of 3% tetracaine. The parietal bone was removed dorsal to the cerebellum and the mesenchymal tissue was withdrawn to expose the cerebellum and the rostral portion of the facial lobes. Electrical activity within the superior secondary gustatory nucleus was recorded extracellularly by glass microelectrodes (2-6 um tip diameter; 1-5 Mohm impedance) filled with 3 M NaCl, 6% fast green FCF in 3 M NaCl, or 2% pontamine sky blue in 0.5 M sodium acetate. Using features of the dorsal surface of the caudal cerebellum as landmarks, the electrode was driven vertically through the cerebellum in a grid of tracks 250 um apart.

Searching for nGS units consisted of systematically testing portions of the oropharyngeal cavity and extraoral body surface with mechanical and chemical stimuli while the electrode was in the vicinity of the nGS. Usually, between 2.3 and 2.7 mm below the surface of the cerebellum, the relatively high level of background activity present in the granular portion of the cerebellum would abruptly cease, and multiunit mechanoresponses would indicate that the

electrode was located in the dorsal region of the nGS. Tactile stimulation was produced by stroking the surface of the fish with a thin glass rod to identify "glide-type" units (Biedenbach, '73). Chemical stimulation consisted of a bovine liver extract solution (10 g/l; gravity filtered), a mixture of amino acids (L-ala, L-arg, L-pro, and D-ala, each at 0.1 mM), and a mixture of nucleotides (adenine, adenosine, AMP, and ATP, each at 10 mM) as search solutions. Individual amino acids were tested once chemoresponsive activity was confirmed. Chemical stimuli were delivered as 1 ml aliquots into a constant water flow (12 ml/min) directed at portions of the body surface or oropharyngeal cavity. The constant water flow allowed for adaptation of the initial mechanical response prior to chemical stimulation. Amplified unit activity was recorded on magnetic tape and subsequently analyzed with a window discriminator (BAK, DIS-1) or a computer unit analysis program (Brainwave) to isolate single unit responses.

Electrode placement was verified histologically by iontophoretic marking with pontamine sky blue (2% in 0.5 M sodium acetate; 10 uAmps of cathodal DC current for 30 minutes) or fast green FCF (2% in 2 M NaCl; 20 uA cathodal current for 30 min). After recording and dye marking, the animal was perfused intracardially with teleost Ringer's solution, followed by 4% glutaraldehyde in 0.1 M phosphate buffer (7.2 pH). The brain was removed and embedded in egg yolk, post-fixed for 4 hours, and placed in a sucrose buffer solution for 12-24 hours (Morita and Finger, '85). The brain was sectioned at 33-50 um on a freezing

microtome in either the transverse or horizontal plane, and the sections were counter-stained with thionin or neutral red (Bures et al., '83).

Anatomy -

Serial sections through the nGS of Nissl-stained material were used for cytoarchitectural analysis of the nucleus. Twenty channel catfish were used for horseradish peroxidase (HRP) labeling of both afferent and efferent nGS connections. Injection sites included the vagal (VL) and facial (FL) lobes, the nGS, and different diencephalic regions known to receive nGS projections (Kanwal et al., '88). Horseradish peroxidase (Sigma type IV; 10% in 0.1 M Tris, pH 8.6) was injected iontophoretically from glass electrodes (2-5 um tip, 3-5 Mohms; 10 uA anodal DC current for 20-50 min) following electrophysiological identification of unit activity. After HRP injection, the removed bone was replaced with dental cement and the fish was placed in an aquarium for recovery. After surviving 48-96 hours, the fish was re-anaesthetized with tricaine methane sulfonate (MS-222, 100 mg/liter), then processed as described above for identification of electrode location following electrophysiological recording. The sections were reacted for peroxidase reactivity with a modified Hanker-Yates protocol (Bell et al., '81) or with tetramethyl benzidine (Mesulam, '78).

RESULTS

Cytoarchitecture -

Nissl-stained sections show that the superior secondary gustatory nucleus (nGS) of Ictalurus punctatus is located ventral to the corpus cerebelli, caudal to the valvula cerebelli, and lateral to the brachium conjunctivum. The nGS is essentially spherical, encapsulated by fiber tracts - medially by the brachium conjunctivum, laterally by the secondary gustatory tract (2G), caudally by fascicles of secondary gustatory neurons running medial from 2G into the nGS, and rostrally by the commissure of the nGS (Fig. II.1).

At the caudolateral margin of the nGS several fascicles of fibers separate from the medial 2G and travel medially into the caudal nGS (Fig. II.1). Each fascicle continues rostrally within the nucleus to extend to middle and rostral regions of the nGS. Rostral to the separation of these fascicles from the 2G, the tract gives off additional fascicles which pass rostromedially through the nGS to cross the midline as the caudal commissural fibers. The 2G continues to the rostrolateral margin of the nGS and curves medially to form the rostral portion of the commissure.

The diagonal fascicles that pass from the 2G to the commissure divide the nGS into two regions, rostrolateral and medial (Fig. II.1). This subdivision is reflected by cell densities within the nucleus. Within the rostrolateral region, large (30-50 um) and medium-sized (15-25 um) cell bodies are concentrated in the rostral portion, immediately caudal to the commissural fibers. The medial region also

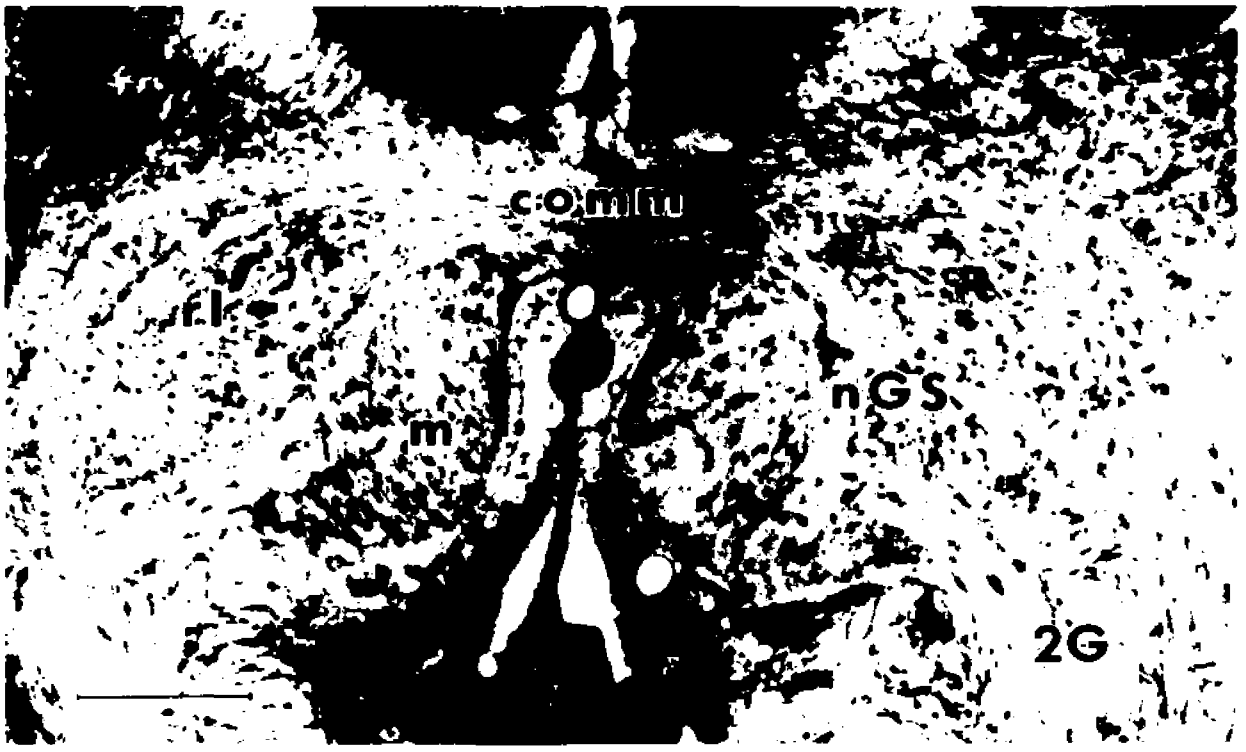


Figure II.1. Horizontal section through the nGS, Nissl-stained with thionin, showing rostrolateral (rl) and medial (m) subdivisions separated by fascicles of the ascending secondary gustatory tract (2G), which form the caudal portion of the commisure of the nGS (comm). Between the medial surface of the nGS and the rostral extension of the fourth ventricle (V) are fibers of the brachium conjunctivum. (Top of figure is rostral; scale bar is 500 um.)

contains a congregation of these cells in the rostral portion, as well as concentrations along the medial and ventral margins of the caudal portion. Along with these localized concentrations, large and medium-sized cells are also sparsely scattered throughout the central region of the nGS. Small neurons (5 um) are located throughout the nGS, including the commissure (see Fig. II.6A).

Electrophysiology -

Twenty-eight single units and thirty-five multiunit records were acquired from the nGS of twenty-eight catfish. Responses were found throughout the nGS (Fig. II.2), but were more evident around the rostral, medial, and ventral margins of the nucleus. The spontaneous activity of thirteen single units that were analyzed for spontaneous behavior ranged from 0-18 spikes/sec, with a mean of 5.6 spikes/sec and a median of 3 spikes/sec. Three units (11%) had no spontaneous activity. Eight (29%) of the single units were bimodal, responding to chemical and mechanical stimuli. The remaining twenty nGS units (71%) responded only to mechanical stimulation. Of the thirty-five multiunit recording sites, only one (3%) responded to tastants. The likelihood of finding taste activity was even lower than these results indicate, since many mechanosensitive units were bypassed for further analysis in an effort to find taste activity. Three single units (11%) and three multiunit responses (9%) were suppressed, while the remaining twenty-five units and thirty-two multiunit responses were facilitated by peripheral mechanical stimulation.

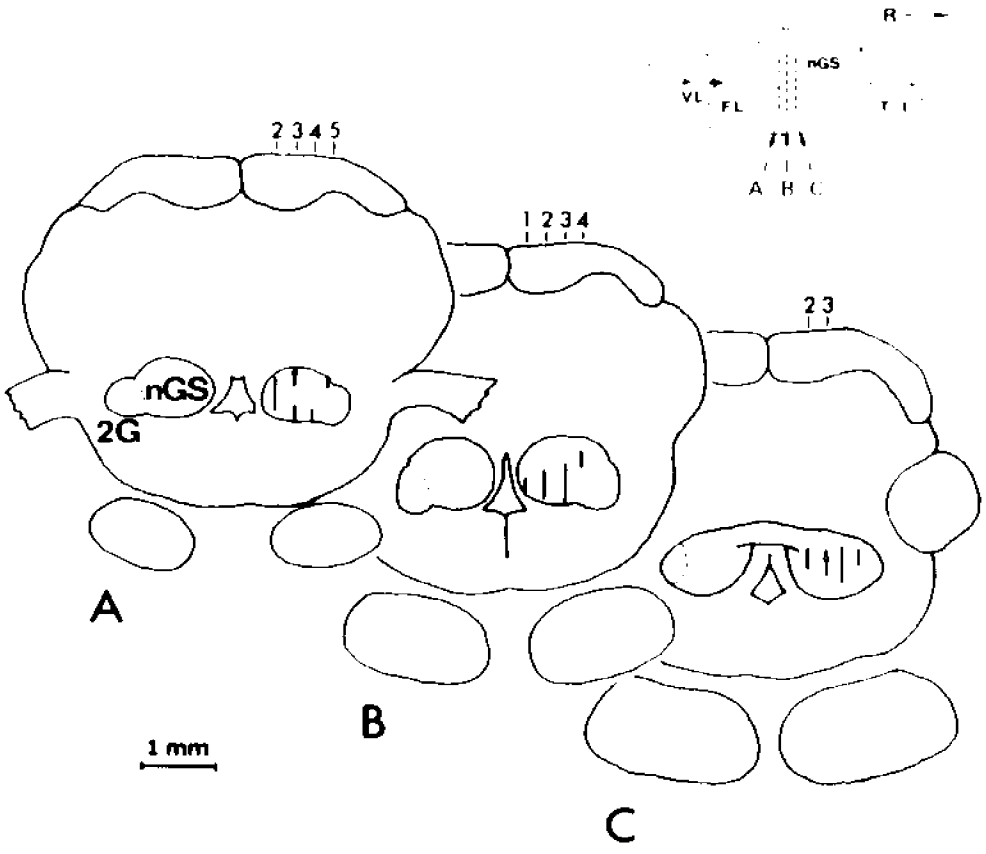


Figure II.2. Schematic diagram of nGS at caudal (A), middle (B), and rostral (C) levels (300 um apart). Vertical lines through the nucleus indicate electrode locations marked after successfully recording unit activity. The circles on electrode tracks in A and C show locations where taste responses were recorded.

Receptive field (RF) sizes of all mechanosensitive nGS neurons and mechanosensitive receptive areas (RAs) of multiunit preparations examined in this study were greater than 100 mm^2, often including both the oropharyngeal cavity and the whole body surface (Table II.1). The most restricted RFs included only the ipsilateral mouth (barbels and lips) from four units (14%), including one in the dorsolateral nGS (Fig. II.3A), and one unit in the dorso-medial nGS responding to stimulation of the ipsilateral oropharyngeal cavity. Most units (61%) were responsive to stimulation of the ipsilateral or bilateral whole body surface. Other units had complex RFs, including the bilateral mouth region and ipsilateral flank, or the oropharyngeal cavity and bilateral flank. Responses to stimulation of the oropharyngeal cavity were found throughout the nGS, often combined with extraoral RF patterns (29% of all units). Because of the large and complex RFs, no distinct topographic arrangement was observed for the mechanosensitive nGS neurons. Even the units with more restricted RFs were not obviously organized within the nGS according to RF pattern.

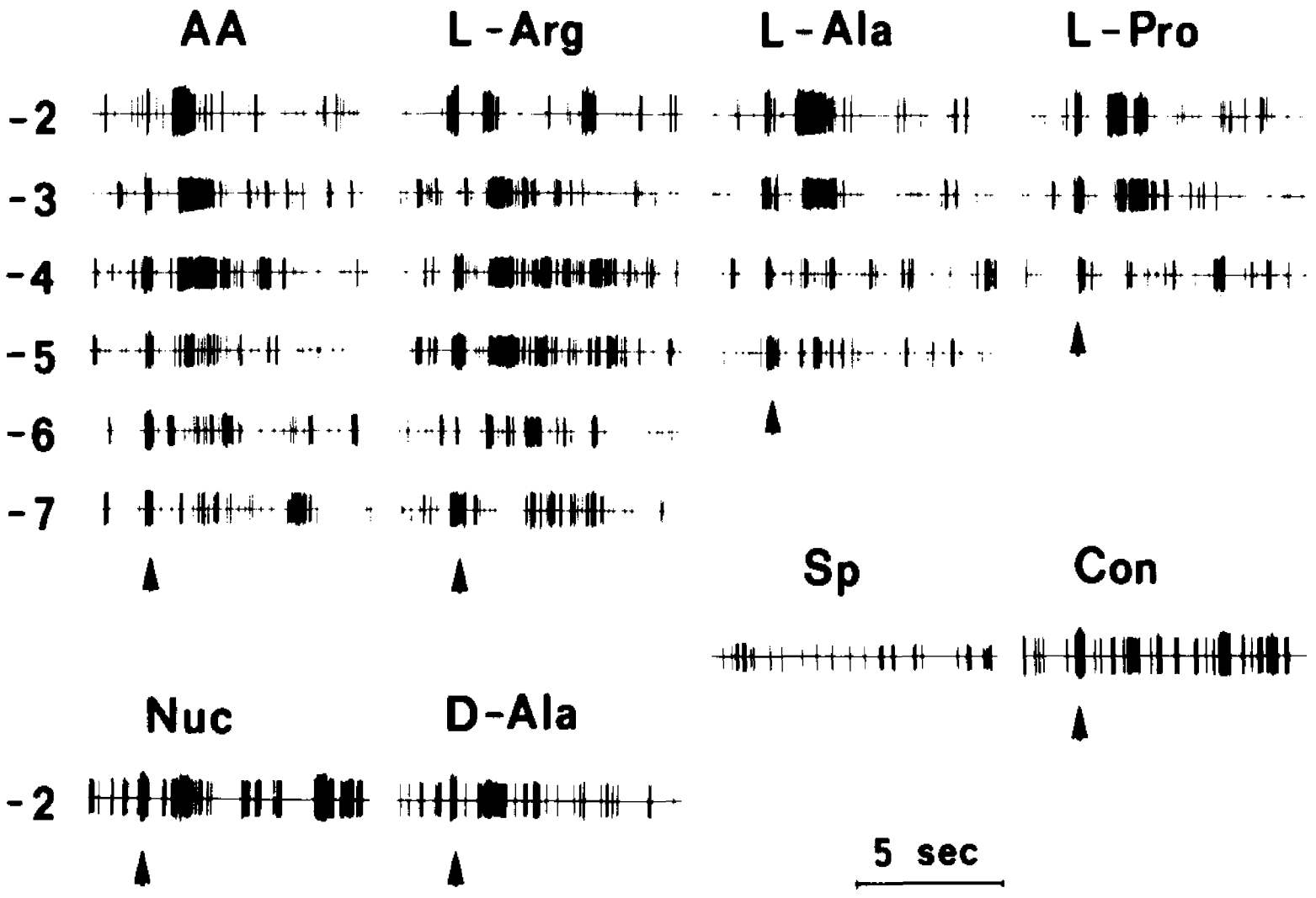
Chemosensitive nGS neurons were more difficult to locate and maintain than were mechanosensitive neurons. Taste units were located within the mechanosensitive areas of the nGS (Fig. II.2), and all of the identified chemosensitive units were bimodal. Six of the eight taste responsive units were facilitated by chemical stimuli (Fig. II.4), whereas the other two were suppressed (Fig. II.5). Taste activity was typically greatest to the mixture of amino acids, then to L-arginine, L-alanine, and L-proline, in order of relative effectiveness. One unit from the medial region of the nGS was tested

Table II.1. Number of single units in the superior secondary gustatory nucleus with mechanoresponses to a given receptive field (number of multi-unit recording sites associated with each receptive field are in parentheses).

Receptive field	Ipsilateral	Contralateral	Bilateral	Total
Mouth (lips & barbels)	4 (3)	0	2 (4)	6 (7)
Head	1 (1)	0	2 (2)	3 (3)
Body	4 (2)	0	5 (4)	9 (6)
Oropharyngeal cavity	1	0	1 (1)	2 (1)
Oral and extraoral	3 (6)	0	5 (12)	8 (18)
Total	13 (12)	0	15 (23)	28 (35)

Figure II.3. Transverse sections of nGS in different fish showing pontamine sky blue deposits after recording unit activity. A) Location of extraoral tactile responses in rostrolateral nGS. B) Location of bimodal tactile unit in medial nGS (see Fig. II.4). (Scale bar is 500 um.)

Figure II.4. Responses of a unit from the medial nGS (see Fig. II.3B) to concentration series of several tastants applied to the ipsilateral maxillary barbel. Initial burst to each stimulus (arrowheads) indicates a tactile response to the pressure change produced by switching the stimulus delivery system from background water to test solution. (AA - amino acid mixture, Con - water control, Nuc - nucleotide mixture, Sp - spontaneous activity; numbers on left indicate exponential power of log molar concentration.)


AA
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-2
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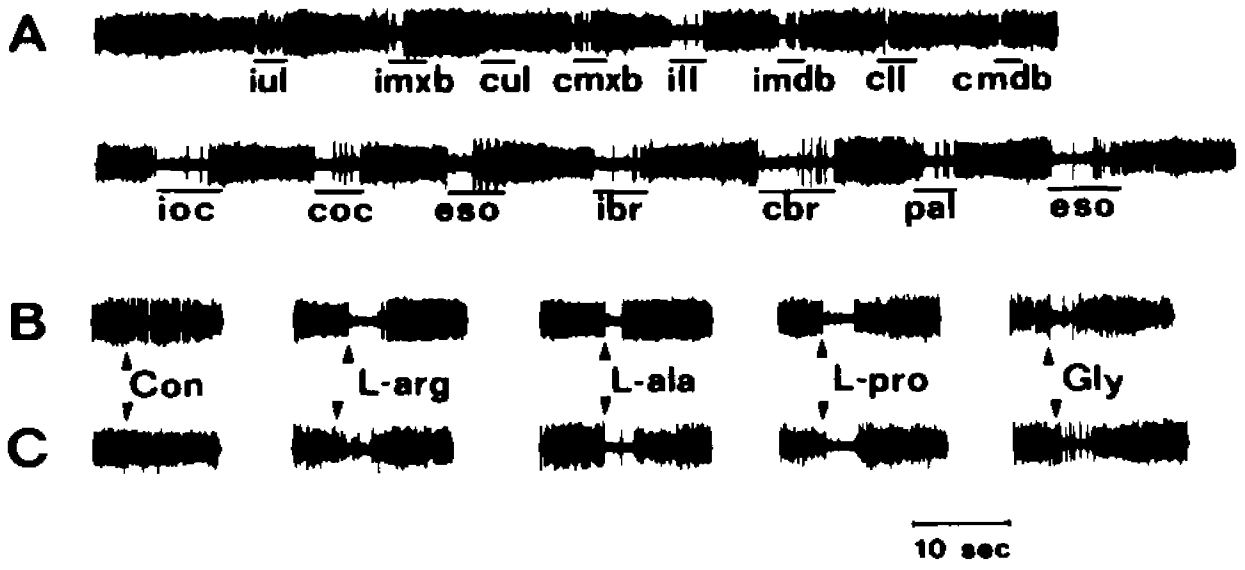


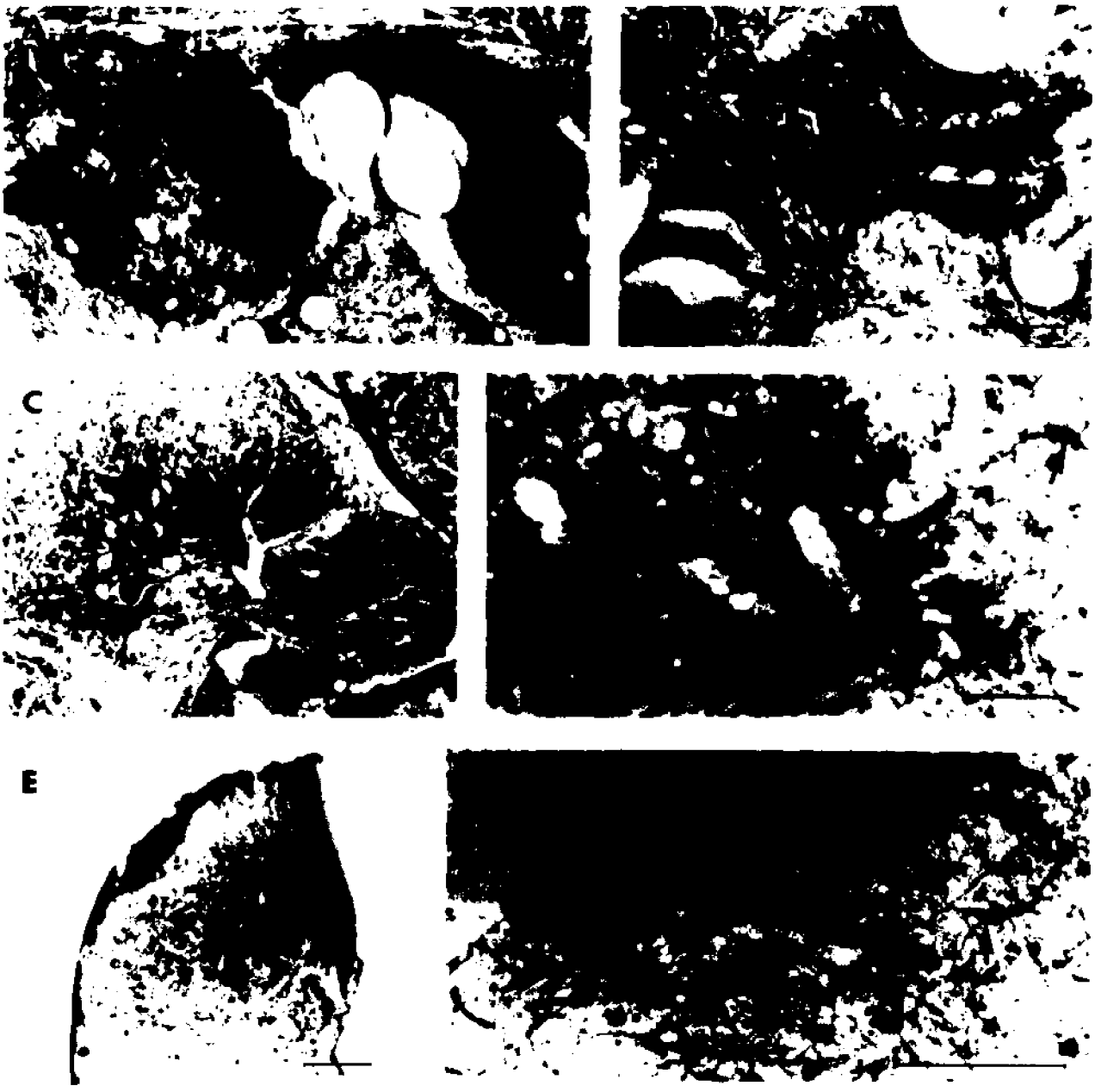
Figure II.5. Responses of a bimodal unit from the central region of the nGS (see Fig. II.2C) to mechanical stimulation of the mouth region (A), and to chemical stimulation of the ipsilateral maxillary (B) and mandibular (C) barbels. (cbr - contralateral gills, cll - contralateral lower lip, cmdb - contralateral mandibular barbel, cmxb - contralatera maxillary barbel, coc - contralateral oral cavity, cul - contralateral upper lip, eso - esophagus, ibr - ipsilateral gills, ill - ipsilateral lower lip, imdb - ipsilateral mandibular barbel, imxb - ipsilateral maxillary barbel, ioc - ipsilateral oral cavity, iul - ipsilateral upper lip, pal - palate, Con - water control, all amino acids are 0.1 mM; arrowheads indicate stimulus onset.)

extensively with the amino acid mixture, L-arg, L-ala, L-pro, D-ala, and a nucleotide mixture (Fig. II.4). Responses from this unit were detected at the following concentrations: amino acid mixture, 1.0 uM; L-arg, 1.0 uM; L-ala, 0.1 mM; L-pro, 1.0 mM; D-ala, 10 mM; and the nucleotide mixture, 10 mM. Another unit from the central nGS was inhibited by tactile stimulation of the oropharyngeal cavity and whole extraoral surface, bilaterally (Fig. II.5A), and by tastants applied to different regions of its RF (Fig. II.5B,C), with responses at 10 uM for L-ala and L-arg, and 0.1 mM for L-pro and gly. Unfortunately, most units could not be maintained long enough for a uniform testing protocol, so a collective analysis of taste responses in the nGS is not yet possible.

Afferent Connections -

HRP injections into the VL, FL, and nGS labeled fibers of the ascending secondary gustatory tract. Restricted injections in the nGS (Fig. II.6A) labeled fibers within the fascicles of the secondary tract that enter the nGS at its caudolateral border (Fig. II.6B) and divide the nucleus into the regions identified above. There was also dense labeling in the contralateral nGS corresponding to the location of the injection site, apparently from fibers of small, intrinsic neurons passing through the commissure of the nGS. Such restricted injections also retrogradely labeled cells in both the VL and FL (Fig. II.2C-E). Small (8-12 um), oval or fusiform cells were labeled throughout the FL (Fig. II.2C). Similar labeling was present in the VL, except for a conspicuous absence of labeled cells in the dorsal

Figure II.6. HRP-labeling of ascending secondary gustatory neurons after an injection into the nGS (A-E), and after a vagal lobe injection (F). A) Iontophoretic injection site in the rostromedial nGS (asterisk) showing retrogradely labeled 2G fibers entering the lateral nGS and orthogradely labeled 3G fibers at the ventromedial border of the nucleus (note the small intrinsic neurons in the commissure and their fibers in the portion of the contralateral nGS corresponding to the injection site). B) Fascicles of 2G fibers separating from the tract to enter the caudal and lateral borders of the nGS. C) Retrogradely labeled cells and their axons scattered throughout the FL. D) Higher magnification of FL cells showing rather typical fusiform-shaped cells with few apical dendrites (arrowhead). E) Labeled cells in the central portion of the VL. F) Scattered fibers in the medial nGS after a VL injection with apparent terminals on processes and somata of nGS neurons (arrowheads). (Scale bars in A,B,C, and E are 250 um, scale bars in D and F are 100 um.)


C
E

cap of the nucleus (Fig. II.2E). The retrogradely labeled VL cells were also small oval cells, often with visible dendritic arborizations covering 100-200 um of the lobe. The proportion of cells labeled in the FL to those in the VL was consistent, regardless of the location of the injection site within the nGS. The number of FL cells varied from 30-70 to 100-250 per 33 um section, while the number of VL cells varied from 10-20 to 50-70 per section. There were few labeled cells found in the FL or VL of the contralateral side and none in the intermediate nuclei of the FL (nIF) or VL (nIV).

Injections into each of the medullary lobes reflected the convergence indicated by nGS injections. Although label from the VL was heavier in the rostrolateral nGS, and FL fibers were more numerous in the medial and caudal nGS, small fibers and apparent terminals from both medullary centers were found throughout the nGS (Fig. II.2F).

DISCUSSION

A notable difference between the response characteristics of nGS neurons reported here and the characteristics of medullary gustatory neurons was the typical receptive field size. Whereas RFs of neurons in the nGS of both the channel catfish (present results) and the carp (Marui, '81) ranged from several cm^2 to the whole body surface, RF size in the FL ranged from 2-100 mm^2 (Peterson, '72; Biedenbach, '73; Marui and Funakoshi '79). Multiunit recordings from the FL of the catfish also showed RFs including only portions of individual barbels or restricted areas of extraoral epithelium (Hayama and Caprio, '89). Units from the nIF of the channel catfish, a relatively small group of large cells ventral to the lobular portion of the FL (Herrick, '06), had RFs up to several cm^2, but the RFs were limited to the anterior head and oral cavity (Marui and Caprio, '82; Hayama and Caprio, '90). In the VL, RFs of single units and multiunit recordings were restricted to adjacent branchial arches or a small portion of the oropharyngeal cavity (Kanwal and Caprio, '88). Neurons in the nGS of the carp were not tested for oral responsiveness, but 29% of the sampled neurons (and 50% of the multiunit recordings) in the nGS of the catfish had RFs that included epithelia innervated by both facial and glossopharyngeal/vagal systems, respectively (Table II.1).

Restricted HRP injections in the nGS and medullary lobes (Fig. II.6) supported the convergence of facial and glossopharyngeal/vagal information within the nGS as indicated by the electrophysiological results. The densest labeling followed the patterns identified

previously in the bullhead (Finger, '78) and carp (Morita et al., '80, '83), with VL efferents located lateral to FL efferents in the secondary tract and terminating lateral to them in the nGS; however, nGS injections throughout the nucleus retrogradely labeled cells in both the FL (similar to the medium-sized cells of the bullhead FL; Finger, '78) and the VL, and medullary injections labeled 2G fibers which appeared to terminate diffusely in all regions of the nGS (Fig. II.6E). These results suggest a convergent termination of efferents from both the VL and FL onto individual nGS neurons throughout the nucleus; however, it is possible that the nGS injection sites could have labeled adjacent 2G fibers either in the fascicles that pass through the nGS or in the commissure. Double-labeling studies involving injections into both medullary lobes and electron microscopic analysis are necessary for confirmation of this hypothesis.

An alternative explanation for the convergence of gustatory input in the nGS could be a result of the large dendritic fields of nGS neurons overlapping both vagal and facial regions of the nucleus (Finger, '88b); however, Golgi analysis showed nGS neurons and their dendrites are contained within clusters throughout the nucleus (Finger, '78). Another possibility is that nIF cells with large RFs (Hayama and Caprio, '90) project to the nGS (Finger, '78); however, RFs of nIF cells included either the extraoral surface or the rostral part of the oral cavity (Hayama and Caprio, '90), and none of the reported RFs included the epithelium of the posterior oropharyngeal

cavity as observed for nGS units. In addition, the RFs of nIF cells were substantially smaller (Hayama and Caprio, '90) than the RFs of nGS neurons reported in this study. Even though negative results from anatomical labeling experiments cannot provide conclusive proof of neural connections, the absence of labeled nIF cells following HRP injections in the nGS (present study) is further evidence that the large RFs of nGS neurons are not due to nIF projections to the nGS. Thus, convergence of input from both FL and VL projections onto individual nGS neurons is most consistent with the present and previous results.

The marked increase in RF size of nGS neurons compared to FL and VL neurons and the lack of an obvious topographical organization in the nGS present a striking contrast to the representational maps prominent in the VL (Braford, '86; Kanwal and Caprio, '87 '88; Finger, '88a) and FL (Peterson, '72; Marui and Caprio, '82; von Bartheld and Meyer, '85; Kiyohara et al., '85, '86; Puzdrowski, '87; Kotrschal and Whitear, '88; Marui et al., '88; Hayama and Caprio, '89, '90) of many teleosts. This difference indicates a functional distinction between medullary gustatory centers and higher nuclei. The continuous spatial maps found in the medulla facilitate spatial filtering and feature extraction (Nelson and Bower, '90) of gustatory stimuli, as proposed for the point-to-point reflexive connections between the palatal organ of the oropharyngeal cavity and the VL of the goldfish (Finger, '88a). The different brainstem connections of the VL and FL of catfishes (Finger and Morita, '85; Morita and Finger, '85) suggest that the behavioral patterns associated with each nucleus (Atema, '71) are

accomplished through separate networks within the caudal brainstem. Evidence from the somatosensory system in mammals (Dykes, '83), and multisensory midbrain of teleosts (Bastian, '82; Heiligenberg, '88), amphibians (Bartels et al., '90), reptiles (Newman and Hartline, '81), birds (Knudsen and Konishi, '78; Knudsen, '82; Konishi, '86), and mammals (Drager and Hubel, '75; King and Palmer, '85; Meredith and Stein, '86; Sparks and Nelson, '87) indicates highly-ordered, topographical maps allow spatial localization of sensory stimuli. It is possible that ascending gustatory information forms a computational map (see Konishi, '86) in the nGS of the channel catfish, but further analyses of the responsiveness of nGS cells to taste and tactile stimulation, as well as the effects of both ascending and descending input to the nGS, are necessary for an identification of the functional organization within this nucleus.

Localization and orientation are often enhanced by multimodal sensory input (Stein et al., '88, '89). The overlapping taste and tactile maps in the FL (Marui and Caprio, '82; Marui et al., '88; Hayama and Caprio, '89) and VL (Kanwal and Caprio, '88) of teleosts can similarly explain early behavioral reports (Herrick, '04; Olmsted, '18; Bardach and Case, '65) that fish use the combination of taste and tactile information to locate and determine the palatability of potential food sources. Behavioral studies on rats (Zeigler et al., '84; Berridge and Fentress, '85) also suggested an important role for the combination of gustation and somatosensation in palatability determination and feeding in mammals. This is supported by recent

psychophysical studies in humans (Lehman, '91), which revealed that localization of an intraoral gustatory stimulus is dependent on its association with concurrent somatosensory stimulation. In catfish, not only do trigeminal afferents overlap facial afferents in the representation of their RFs in the FL (Kiyohara et al., '86), but fibers responsive to mechanosensory stimulation also are present in facial (Biedenbach, '71; Davenport and Caprio, '82), as well as the glossopharyngeal and vagal (Kanwal and Caprio, '83) nerves of the catfish. The prominent association of taste and tactile responsiveness in peripheral and central electrophysiological studies suggests that the gustatory system in teleosts is a taste/tactile system involving the integration of these two senses.

The absence of an identifiable map in the nGS of ictalurid catfish poses an intriguing question as to the functional importance of the taste/tactile information ascending from the hindbrain. Efferents from the nGS enter the diencephalic inferior lobes (Herrick, '05; Shanklin, '35; Barnard, '36; Morita et al., '80, '83; Kanwal et al., '88; Wullimann, '88) where they terminate in the region of cells that project (a) back to the medullary gustatory nuclei (Luiten and van der Pers, '77; Finger, '78; Morita et al., '83; Morita and Finger, '85), and (b) through the medial and lateral forebrain bundles to the telencephalon (Echteler and Saidel, '81; Murakami et al., '86; Airhart, '87; Kanwal et al., '88; Striedter, '90b). Further analyses of the specific connections of nGS efferents, as well as the responses of their postsynaptic cell groups, are needed to determine the

functional significance of the ascending gustatory pathways (see Chapters III and IV).

The pathway of gustatory information from the facial and vagal lobes to the nGS of fish is remarkably similar to the mammalian connections between the nucleus of the solitary tract and the parabrachial nucleus (Norgren and Leonard, '73). More specifically, the teleost 2G resembles the secondary tract of rodents (Norgren, '78; Travers, '88; Herbert et al., '90) rather than that of monkeys (Beckstead et al., '80), which carries primarily visceral information from the nST to the PBn. Gustatory information in monkeys is relayed directly from the nST to the ventral posteromedial thalamic nucleus (Beckstead et al., '80). Electrophysiological studies in the PBn of the rat indicated an increased convergence of facial gustatory information with glossopharyngeal (Norgren and Pfaffmann, '75; Ogawa et al., '82; Hayama et al., '87; Nishijo and Norgren, '90) and vagal (Hermann and Rogers, '85) input, compared to the nST (Ogawa and Hayama, '84). An overlap of gustatory and visceral information is also apparent in the hamster PBn, where neurons in both the gustatory and visceral regions of the PBn respond to taste stimulation of the anterior tongue (Halsell and Frank, '91). Bimodal (taste/tactile) gustatory neurons are also more prominent in the PBn and gustatory thalamus than in the nST (Ogawa et al., '87), suggesting the importance of mixed chemical and mechanical detection in the higher gustatory projections of mammals. Unfortunately, too little is presently known about the gustatory-visceral relationships in the teleost gustatory system, or about the effects of input from higher

centers, for a direct functional comparison with much of the mammalian data.

Clues to the functional role of a given nucleus within a CNS pathway can sometimes be deduced from electrophysiological characteristics of neurons within that nucleus. In the present study, spontaneous behavior and relative amounts of suppression of nGS neurons were identified for a comparison with what was previously reported from other teleost gustatory centers. In an early study of FL units in the goldfish, Peterson ('72) found few or no spontaneously active cells and, thus, no inhibition to tactile stimulation of the peripheral RFs. This contrasts with a report of chemosensitive cells in the medullary gustatory nuclei of the carp (Vasilevskaya and Polyakova, '78), where only 10% of the sampled cells in the FL and 50% of those in the VL had no spontaneous activity. Recent studies of the FL (Hayama and Caprio, '89) and nIF (Hayama and Caprio, '90) in the channel catfish identified more suppression of nIF units than FL units, but the amounts were not quantified. In the VL of the channel catfish, Kanwal and Caprio ('88) found mean spontaneous rates of 1.7 spikes/sec for chemoresponsive neurons and 3.9 spikes/sec for mechanoresponsive neurons, with 20% of their sampled units having no spontaneous activity. Also found was that 15% of both mechanoresponsive and chemoresponsive VL units were suppressed by stimulation. The results of the present study, indicating a mean activity rate for nGS units of 5.6 spikes/sec and no spontaneous activity in 23% of the sampled units, are quite similar to those

reported for the VL of the catfish. There were slightly fewer nGS units inhibited than reported in the VL, but this difference is probably due to the small number of nGS neurons sampled. Neurons in the primary and secondary gustatory nuclei display apparently simple response patterns to mechanical and chemical stimulation of their RFs, suggesting that the nGS operates as a relay center for gustatory information traveling from the medulla to higher centers. The only major difference observed in the response patterns between the medullary taste nuclei and the nGS of catfish is the increased RF size of nGS neurons, including the convergence of facial and vagal information.

The responsivity of peripheral taste nerves to amino acids was studied in catfish (Caprio, '82; Davenport and Caprio, '82; Kanwal and Caprio, '83; Kanwal et al., '87), carp (Marui et al., '83b), rainbow trout (Marui et al., '83a), eel (Yoshii et al., '79), and other teleost species (see Caprio, '84). There is, however, little information regarding the processing of this information centrally. Relatively few neurons responsive to taste stimulation were found in the nGS (present report), VL (Kanwal and Caprio, '88), and FL (Biedenbach, '73; Marui and Caprio, '82; Marui et al., '88; Hayama and Caprio, '89, '90) of silurids. The present study is the first to report dose-dependent taste responses to amino acid concentrations approaching those found effective in peripheral electrophysiological studies (Caprio, '75; Davenport and Caprio, '82; Kanwal and Caprio, '83). Other studies reporting taste activity in the medulla (Marui, '77; Vasilevskaya and Polyakova, '78, '80) and nGS (Marui, '81) of the

carp tested classical taste stimuli (acids, salts, and sugars), which are not as effective as taste stimuli in teleosts as are amino acids (Caprio, '84, '88; Davenport and Caprio, '82). The present results show that neurons in the nGS of catfish respond to amino acid stimuli applied to the oral cavity and the extraoral surface; however, further analyses of taste responses to amino acid stimuli in the medullary and isthmic gustatory nuclei of catfish are required to determine whether unit taste specificities and response properties differ centrally from those determined from peripheral nerve recordings (Davenport and Caprio, '82; Kohbara et al., '90; Wegert and Caprio, '91).

In summary, the exquisite topographical representation displayed by the somatotopic map of the body surface onto the FL and the viscerotopic map of oropharyngeal structures onto the VL of the channel catfish is not maintained at the next synaptic level within the nGS. Further, the large RFs of nGS units include both oral and extraoral structures of the catfish, indicating the convergence of VL and FL efferents onto nGS neurons. These findings suggest that the functional role of the nGS of catfish differs from the reflexive stimulus localization and orientational roles proposed for the topographically organized medullary gustatory nuclei.

Chapter III

Diencephalic Gustatory Connections

INTRODUCTION

Vertebrate gustatory pathways ascend from the primary sensory nuclei for the facial, glossopharyngeal, and vagal nerves in the medulla to a secondary gustatory nucleus in the metencephalon and to a number of nuclei in the forebrain. Along with these secondary projections, there are tertiary connections between the metencephalic nucleus and the forebrain. The primary gustatory nucleus in mammals is the rostrolateral portion of the nucleus of the solitary tract (nST), and its projections differ somewhat between the mammalian groups studied. In rodents, the gustatory efferents from the nucleus of the solitary tract terminate solely in the parabrachial nucleus (PBn) of the pons (Norgren and Leonard, '73; Norgren, '78; Travers, '88), while the nST of primates sends gustatory information to the parvicellular portion of the ventral posteromedial thalamic nucleus (VPMpc) and visceral information to the parabrachial nucleus (Beckstead et al., '80).

The parabrachial nucleus of mammals has two major ascending projections, one to the ipsilateral and contralateral VPMpc (Norgren and Leonard, '73) and the other to the lateral hypothalamus (LH), central nucleus of the amygdala (CAn), and bed nucleus of the stria terminalis (BST; Norgren, '76). There are also less dense projections of parabrachial efferents to other thalamic, hypothalamic, and ventral forebrain nuclei (Saper and Loewy, '80), as well as overlapping secondary projections from the general visceral portion of the caudal nucleus of the solitary tract to these forebrain regions (Ricardo and

Koh, '78). Gustatory VPMpc efferents ascend to the agranular insular cortex (Norgren and Wolf, 1975), while the lateral hypothalamus, central nucleus of the amygdala, and bed nucleus of the stria terminalis send descending efferents to the nucleus of the solitary tract and parabrachial nucleus (van der Kooy et al., 1984).

Comparative studies of the taste system in non-mammalian vertebrates have primarily involved ostariophysine teleosts (silurids, cyprinids, etc.) because of the extensive peripheral distribution of taste buds (Herrick, '01; Atema, '71; Kiyohara et al., '85) and the greatly enlarged medullary gustatory nuclei (Herrick, '06; Evans, '31) of these fishes. The primary gustatory center in the medulla of silurid teleosts, corresponding to the rostrolateral nTS of mammals, consists of the vagal (VL) and facial (FL) lobes (Herrick, '06). Efferents from the vagal and facial lobes ascend in the secondary gustatory tract (2G) to the isthmic region to terminate bilaterally in the superior secondary gustatory nucleus (nGS; Herrick, '05; Finger, '78; Morita et al., '80, '83). Some facial lobe efferents also ascend to the posterior inferior lobe (LI) in the ventral diencephalon (Finger, '78; Kanwal et al., '88). Efferents from the superior secondary gustatory nucleus project in the tertiary gustatory tract (3G) to the posterior inferior lobe (Herrick, '05; Morita et al., '80, '83; Finger, '83; Lamb et al., '87; Kanwal et al., '88; Wullimann, '88).

Little is known about the connections of neurons in the posterior portion of the inferior lobe of teleosts. The nucleus

lobobulbaris (nLB) is a group of large neurons in the vicinity of the diencephalic projections from the facial lobe and secondary gustatory nucleus. Efferents from the nucleus lobobulbaris in catfish (Finger, '78; Morita and Finger, '85; Lamb et al., '87; Kanwal et al., '88) and from nuclei in the posterior inferior lobe of carp (Morita et al., '83) descend to the facial and vagal lobes. Other cells in the posterior inferior lobe of the carp project to the motor nuclei of the trigeminal and facial nerves (Luiten and van der Pers, '77). Neurons in this region of the diencephalon also project to the nGS (Lamb et al., '87; Kanwal et al., '88) and to the telencephalon (Airhart, '87; Kanwal et al., '88; Striedter, '90b).

One problem confounding the analysis of nuclei in the diencephalon of fishes is the morphological diversity of nuclei in this region between different taxa, as well as the confusion in nomenclature resulting from this diversity (see Braford and Northcutt, '83). Recent analyses of the diencephelon of catfish (Striedter, '90a,b) and of actinopterygians in general (Braford and Northcutt, '83) have emphasized the value of studying the differential connections of various sensory systems in proposing homologues for diencephalic nuclei. The present study is an anatomical investigation of the connections of the diencephalic cell groups receiving secondary and tertiary gustatory projections in the channel catfish, Ictalurus punctatus, to characterize morphologically these diencephalic nuclei and to compare the higher gustatory projections of catfish to known gustatory paths in other vertebrates.

MATERIALS and METHODS

Hodological experiments -

Thirty-seven channel catfish, weighing from 10 to 110 g and with standard lengths from 11 to 21 cm, were immobilized with intramuscular injections of Flaxedil (gallamine triethiodide, 0.5 mg/kg) and secured in a Plexiglass container. The gills and oral cavity were perfused with aerated, charcoal-filtered city tap water (artesian well water), and supplemental doses of Flaxedil were administered as required. The dorsal surface of the head was anaesthetized by topical application of 3% tetracaine. The parietal bone was removed dorsal to the cerebellum and the mesenchymal tissue was withdrawn to expose the cerebellum, the caudal portion of the telencephalon, and the rostral portion of the facial lobes.

Horseradish peroxidase (Sigma type IV; 10% in 0.1 M Tris, pH 8.6) was injected iontophoretically from glass electrodes (2-5 um tip, 3-5 Mohms; 10 uA anodal DC current for 20-50 min) after identifying unit activity within the vagal lobe, facial lobe, superior secondary gustatory nucleus, caudal inferior lobe, and torus semicircularis (TS), respectively. Additional injections into the vagal lobe, facial lobe, and medial telencephalon (Tel) were applied as an HRP paste (in Tris buffer) on the tip of an insect pin (size 00). Four fish received injections in the vagal lobe, eight in the facial lobe, four in the secondary gustatory nucleus, fifteen in the inferior lobe, two in the torus semicircularis, and four in the medial telencephalon.

After HRP injection, the removed bone was replaced with dental cement and the fish was placed in an aquarium for recovery.

After surviving 2-7 days, the fish was re-anaesthetized with tricaine methane sulfonate (MS-222, 100 mg/liter) and perfused intracardially with teleost Ringer's solution followed by 4% glutaraldehyde in 0.1 M phosphate buffer (7.2 pH). The brain was removed and embedded in egg yolk, post-fixed for 4 hours, and placed in a sucrose buffer solution for 12-24 hours (Morita and Finger, '85). The brain was sectioned at 33-50 um on a freezing microtome in either the transverse, sagittal, or horizontal plane, and the sections were reacted for peroxidase reactivity with a modified Hanker-Yates protocol (Bell et al., '81) or with tetramethyl benzidine (Mesulam, '78). The reacted sections of each brain were mounted in two alternating series; one series was counter-stained with thionin (Bures et al., '83), while the other remained unstained.

Nissl-stained nuclear analysis -

Two additional fish (one with an 18.5 cm standard length and weighing 85.5 g, the other 11.8 cm and 18.8 g) were perfused and sectioned (50 um) as described above to identify the location, size, and cellular morphology of each nucleus. Alternating serial sections were mounted in two series and counterstained with thionin or neutral red, respectively (Bures et al., '83). To eliminate the potential for confusion of the nuclear boundaries between the tightly packed cell groups in the posterior tuberculum, these findings were compared with the HRP results to differentiate the individual nuclei. The

nomenclature used in the present report for the cell groups in the catfish posterior tuberculum is based on previous reports by Striedter ('90a) and Braford and Northcutt ('83).

RESULTS

Nuclear organization -

The posterior tuberculum and caudal hypothalamus of the channel catfish are diencephalic regions located within the caudal portion of the inferior lobe. Five diencephalic nuclei are characterized by connections with hindbrain gustatory centers (see below): the nucleus centralis of the inferior lobe (nCLI) and nucleus diffusus of the inferior lobe (nDLI) in the caudal hypothalamus, and the nucleus lobobulbaris (nLB), nucleus of the lateral thalamus (nLT), and nucleus subglomerulosus (nSG) in the posterior tuberculum. The descriptions of the locations and sizes of these nuclei (Table III.1) are based on measurements from an 18.5 cm specimen, using the most posterior connection between the inferior lobe and the ventral mesencephalic tegmentum (Figs. III.1A, III.2A) as the antero-posterior reference point (LI-0). Transverse sections through this location are also characterized by fascicles of internal arcuate fibers (af) passing transversely along the ventral margin of the mesencephalon and by oculomotor fibers that exit the brain between the inferior lobe and the mesencephalon. Two reference lengths were determined in two Nissl-stained specimens to allow a comparison of the nuclei described in the present study with other diencephalic structures. In an 18.5 cm specimen, the distance from LI-0 to the communication between the third ventricle and the lateral recess of the third ventricle was 1300 um, and the distance from LI-0 to the stalk of the infundibulum was 1700 um. Although the standard length of the smaller Nissl-stained

Table III.1. Description of gustatory nuclei in the inferior lobe of the channel catfish. (Distances are based on a fish with a standard length of 18.5 cm; the 0 pt is the caudal attachment of the inferior lobe to the mesencephalic tegmentum; see text for abbreviations.)

Nucleus		Size (um) (h)x(w)x(l)	Location (um from 0 pt)	Cell Sizes
nCLI:	ltfLI-	250x250x700	-650 to +50	15-20 um (multipolar), 5-10 um (spherical)
	rostral-	600x1200x750	+50 to +800	10-20 um (multipolar), 5-10 um (spherical)
nDLI:		400x700x1000	+200 to +1200	5-10 um (spherical)
nLB:	caudal-	200x750x300	+200 to +500	15-40 um (multipolar)
	rl nLB-	250x250x250	+500 to +750	20-30 um (fusiform)
	nLBp-	200x600x600	-50 to +550	10-15 um (oval), 15-20 um (fusiform)
nLT:		350x250x350	+400 to +750	10-20 um (fusiform), 5-12 um (oval)
nSG:		250x250x250	+650 to +800	8-12 um (spherical)

Reference distances:

connection of 3rd vent. and lat. recess - +1300 um

stalk of infundibulum - +1700 um

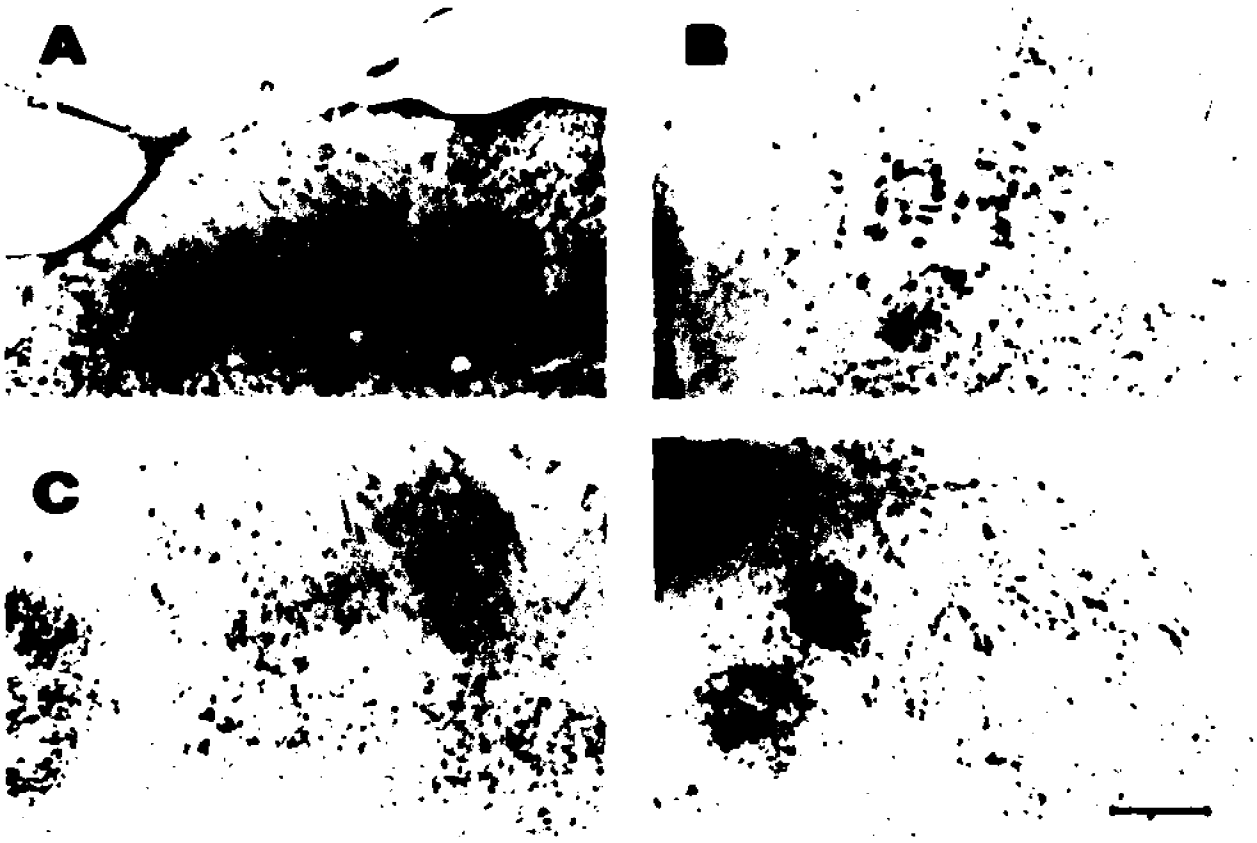


Figure III.1. Nissl-stained transverse sections at four successive levels through the posterior diencephalon of the catfish, corresponding to the nuclear boundaries identified in Fig. III.2. A) LI-0 +50 um: The posterior extent of the connection between the LI and the mesencephalon showing the caudal portions of nCLI and the internal arcuate fibers of the mesencephalic tegmentum. B) LI-0 +250 um: A section through the caudal nLB which is bordered dorsally by the 3G and nLBp and ventrally by the nCLI. C) LI-0 +450 um: A section through the rostral nLB at the level where the 3G separates it into medial and lateral components. The caudal cells of the nLT are seen within the 3G. D) LI-0 +650 um: A section through the nSG, nLT, and rl nLB showing the separation of nLT cells into clusters by fascicles of the 3G. (Scale bar is 250 um.)

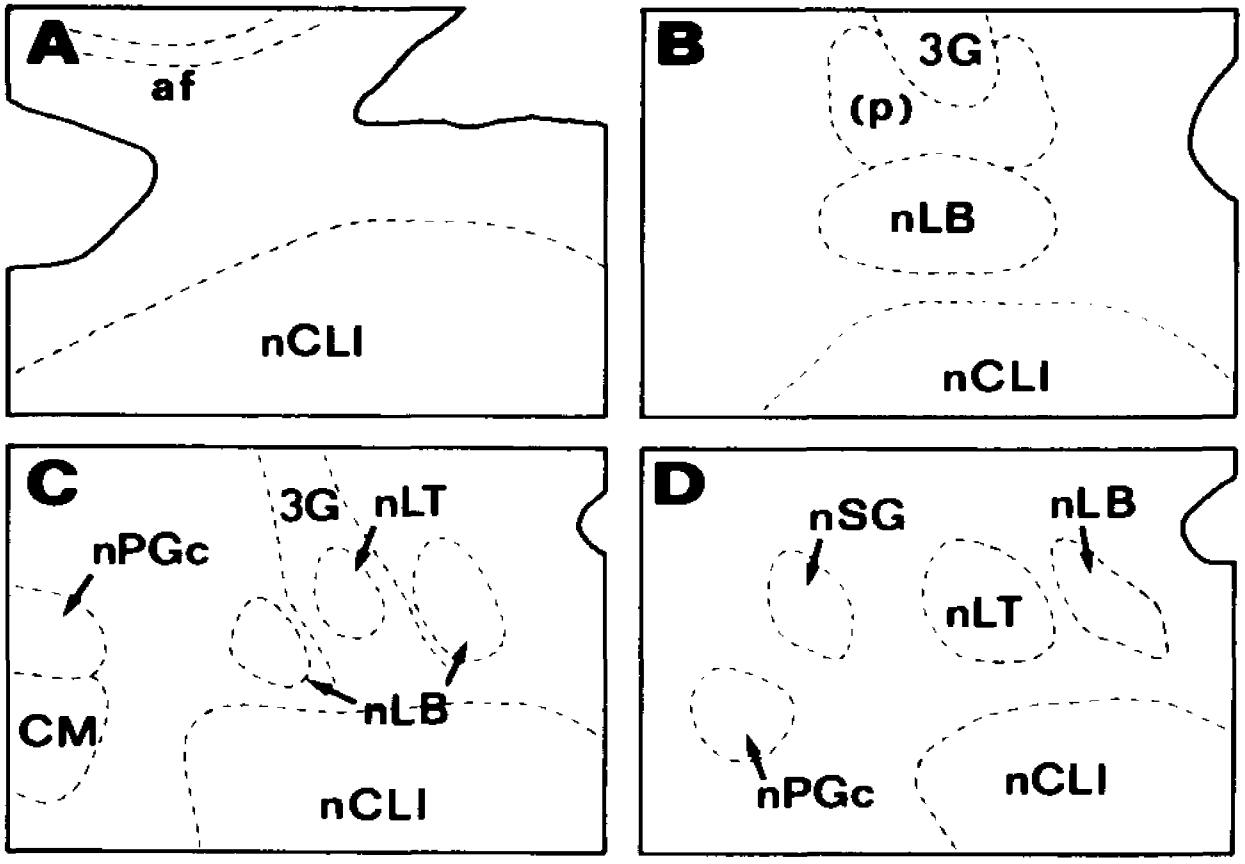


Figure III.2. A schematic diagram of the cell groups corresponding to the sections in Fig. III.1. Identified are the mesencephalic arcuate fibers (af), nucleus centralis (nCLI), tertiary gustatory tract (3G), nucleus lobobulbaris (nLB; p - parvicellular portion), lateral thalamic nucleus (nLT), commissural portion of the preglomerular nucleus (nPGc), corpus mammillare (CM), and nucleus subglomerulosus (nSG).

specimen was 36% less than that of the larger specimen, and both of the rostral diencephalic measurements were approximately 15% less in the 11.8 cm fish, the measurements from LI-0 to all of the nuclei in the posterior tuberculum never varied by more than 50 um between the different fish. The sizes and locations described below were all obtained from the 18.5 cm specimen.

Nucleus centralis - The nucleus centralis is a large nucleus occupying the central portion of the inferior lobe, which is bordered by the medial and lateral nucleus diffusus on either side, by the nucleus lobobulbaris and lateral thalamic nucleus dorsally (Figs. III.1B-D, III.2B-D) and by the nucleus of the lateral recess (nRL) ventrally. The caudal extent of nucleus centralis, approximately 250 um in height by 1000 um in width by 700 um in length, fills the dorsal half of the inferior lobe caudal to its attachment to the mesencephalon (Figs. III.1A, III.2A). Rostral to the attachment of the inferior lobe to the mesencephalon, the nucleus centralis increases in size to 600 um (h) by 1200 um (w), and extends 800 um rostral to LI-0. There are two cell types in the nucleus centralis: small (5-10 um) spherical cells and medium-sized (10-20 um) multipolar cells. Both cell types were found throughout the nucleus centralis, but the caudal portion contained predominantly small cells while the rostral portion had more medium-sized cells.

Nucleus diffusus - The ventromedial and lateral edges of the inferior lobe in the region of the posterior tuberculum contain the small (5-10 um) spherical cells of the nucleus diffusus. The lateral portion of nDLI is larger than the medial portion and fills the whole

lateral extension of the inferior lobe (500 um high by 700 um wide). The two portions of nDLI are separated by the lateral recess and the nucleus of the lateral recess.

Nucleus lobobulbaris - Dorsal to the rostral portion of nucleus centralis and rostral to the junction of the inferior lobe and mesencephalon are the large (15-40 um) multipolar cells of the nucleus lobobulbaris (Figs. III.1B, III.2B). The caudal portion of nLB is 200 um high and 750 um wide, and extends from LI-0 +200 um to +500 um. At approximately LI-0 -450 um, the fibers of the tertiary gustatory tract (3G) pass ventrally from the mesencephalon into the inferior lobe, separating the nucleus lobobulbaris into medial and lateral cell groups (Figs. III.1C, III.2C). Both medial and lateral cell groups consist of medium-sized oval (10-15 um) and fusiform (15-20 um) cells associated with the medial and lateral edges of 3G, respectively. These groups of medium-sized cells (nLBp) continue caudally with the 3G into the mesencephalon (100 um caudal to LI-0), where they surround the ventral edge of 3G. Rostral to LI-0 +500 um, the lateral portion of nucleus lobobulbaris (rl nLB) continues as a compact group of large (20-30 um) fusiform cells with their somata oriented from dorsomedial to ventrolateral (Figs. III.1D, III.2D). This rostrolateral portion of nucleus lobobulbaris continues to LI-0 +750 um.

Nucleus of the lateral thalamus - Adjacent to the rostral edge of the nucleus lobobulbaris, immediately medial to rl nLB, is the lateral thalamic nucleus, which is composed of small (8-12 um) oval and medium-sized (10-20 um) fusiform cells forming an oval nucleus

measuring 350 um (h) x 250 um (w) and extending from LI-0 +400 um to + 750 um (Figs. III.1C-D, III.2C-D). The lateral thalamic nucleus lies within the 3G and is separated by small fascicles of 3G fibers into small groups of cells oriented parallel to the 3G fibers (Fig. III.1D).

Nucleus subglomerulosus - Approximately 250 um medial to the rostral portion of the lateral thalamic nucleus is the spherical (250 um diameter) nucleus subglomerulosus (Figs. III.1D, III.2D), a compact nucleus consisting of small (8-12 um) spherical cells. The nucleus subglomerulosus is 250 um dorsolateral to the commissural preglomerular nucleus (nPGc) and extends from LI-0 +650 um to +800um, where it abuts the caudal margin of the medial preglomerular nucleus (nPGm).

HRP-labeling experiments -

Connections of the tertiary gustatory tract - HRP injections throughout the nGS labeled fibers that collect at the ventral surface of the nucleus to form the ventrolaterally directed 3G. The 3G passes medial to the lateral lemniscus as it continues rostrally through the mesencephalic tegmentum toward the posterior tuberculum. At the level of the nucleus lobobulbaris, the 3G fibers lie immediately dorsal to the large nLB cells (Fig. III.3A). Most of the labeled fibers continue rostrally to the lateral thalamic nucleus where they pass through the nucleus and continue either ventrally to the nucleus centralis or ventrolaterally to a restricted portion of the ventrolateral nucleus diffusus (Fig. III.3A). The 3G fibers that

Figure III.3. Transverse section through the LI at the caudal nLB (LI-0 +300 um) after HRP injection into the nGS. A) Low magnification of the whole LI showing the rostrally directed 3G fibers dorsal to nLB and the descending fibers within the nCLI and nDLI (lateral surface of LI). B) Higher magnification of section A) showing fine fibers leaving 3G to terminate on nLB cells (arrows). (Scale bars in both A) and B) are 200 um.)

enter the nucleus centralis turn caudad and continue into the posterior inferior lobe as the horizontal columns of the ltfLI (see Fig. III.6), with dense terminals throughout both the nucleus centralis (Fig. III.3A) and ltfLI. The labeled ltfLI are 250 um in cross-sectional diameter and extend caudally from LI-0 +50 um to LI-0 -650 um. The 3G fibers that enter the nucleus diffusus continue rostrally from LI-0 +200 um to LI-0 +1200 um, terminating in a 250 um diameter longitudinal column adjacent to the ventrolateral surface of the inferior lobe (Fig. III3A). Some 3G fibers leave the main tract to terminate near the large cells of the caudal nucleus lobobulbaris (Fig. III.3B). The projection pattern of 3G fibers to the inferior lobe was dependent on the location of the injection site in the nGS. Injections into the central region of the nGS labeled fibers in both ltfLI and nucleus diffusus, while medial injections labeled 3G fibers in the ltfLI more densely than fibers in the nucleus diffusus, and lateral nGS injections labeled the rostrolateral projection to the nucleus diffusus more heavily than the ltfLI. Injections into the nGS retrogradely labeled both small and medium-sized cells of the lateral thalamic nucleus, which were surrounded by labeled 3G fibers. Fibers from the labeled cells of the lateral thalamic nucleus projected to the nGS within the 3G.

Medullary connections with the inferior lobe - Injections into both the VL and FL retrogradely labeled the large cells of the nucleus lobobulbaris. Injections in the VL labeled the cells of the rl nLB (from LI-0 +500 um to +750 um), which were located lateral to the lateral thalamic nucleus. These rl nLB cells had a characteristic

fusiform shape, with one or two primary dendrites extending ventrolaterally into the nucleus diffusus (Fig. III.4A). Following FL injections, the large multipolar cells (Fig. III.4B) of the caudal nucleus lobobulbaris were filled (from LI-0 +200 um to +500 um). When densely filled, these cells displayed dendritic fields that spread for several hundred microns across the nucleus lobobulbaris, extending rostrally to the nucleus subglomerulosus (Fig. III.4B) and ventrally into the underlying nucleus centralis (Fig. III.5). Fibers from both nLB regions collected in the lobobulbar tract (tLB), which passed caudally from the inferior lobe ventral to the 3G and continued back to the medullary lobes ventral to the secondary gustatory tract until they turned dorsally with the secondary fibers into their respective lobes.

Along with the heavy projection to the nGS, some of the secondary fibers labeled after FL injections left the 2G and bypassed the nGS, continuing medial to the 3G to terminate in the nucleus subglomerulosus. Some of these fibers bifurcated near the nucleus subglomerulosus to send collaterals rostrally into the nSG and ventrally into the nucleus lobobulbaris (Fig. III.4B). There were no apparent direct projections from the VL into the inferior lobe.

Injections in the inferior lobe - Fifteen fish received HRP injections in different portions of the caudal inferior lobe from LI-0 -600 um to LI-0 +700 um. The results from different injection sites are grouped here by region: caudal nucleus centralis (ltfLI), medial nCLI, and lateral nCLI.

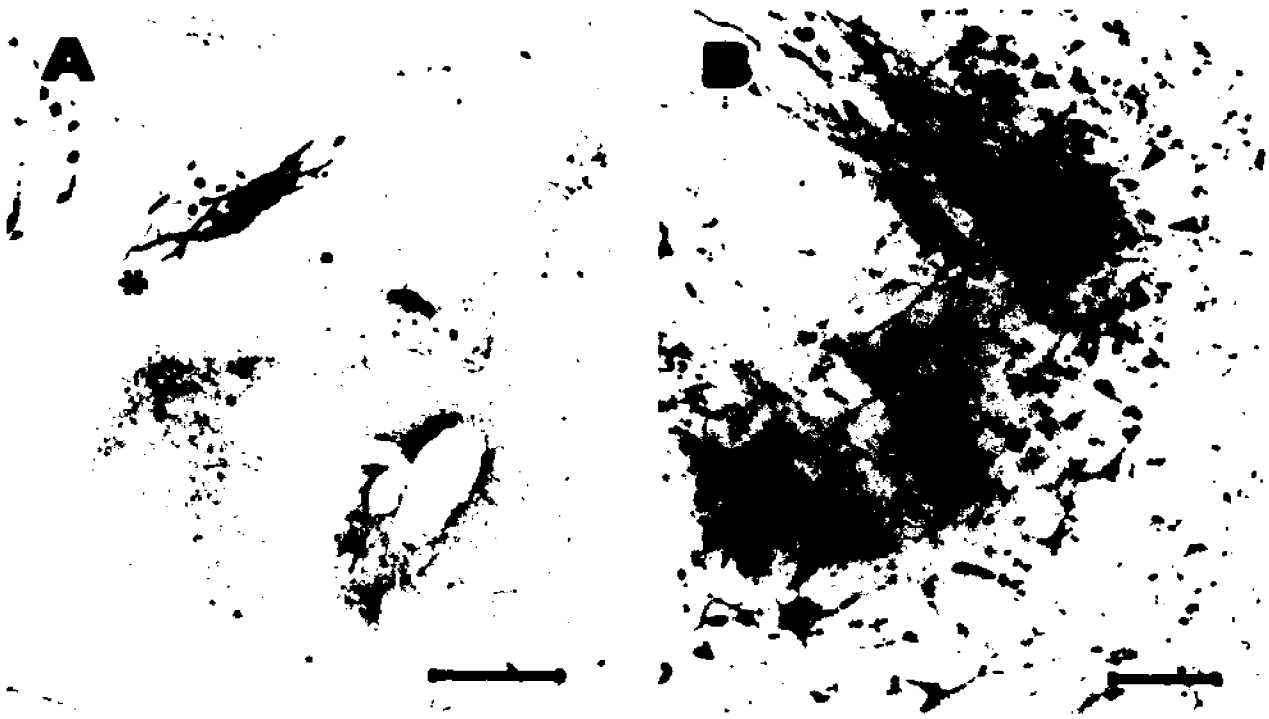
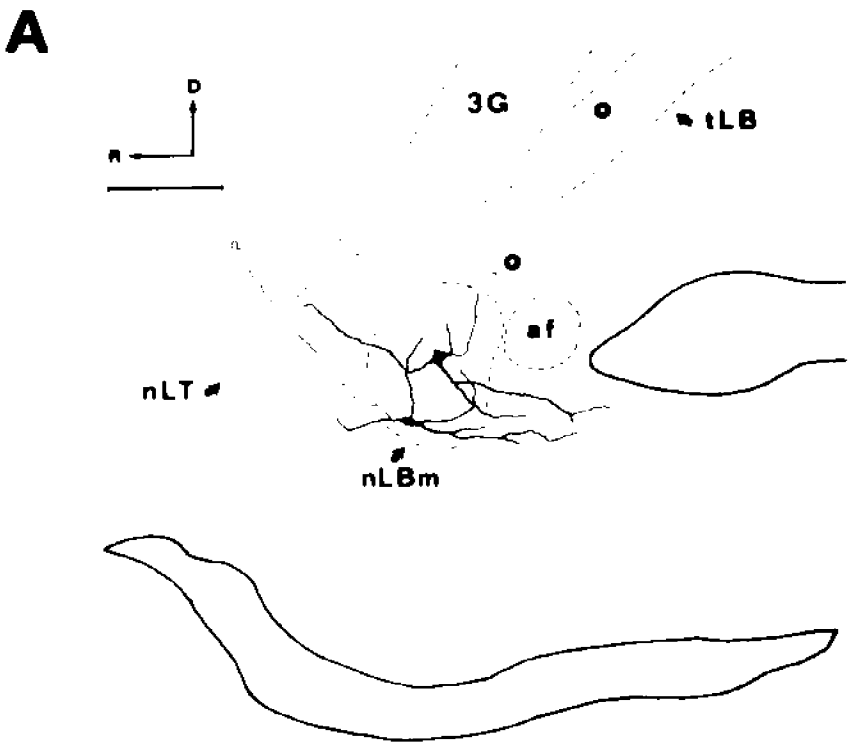


Figure III.4. Retrogradely labeled somata in the nLB after HRP injections into the medullary gustatory nuclei. A) Transverse section of fusiform cells in the rl nLB (LI-0 +650 um) labeled after injection in the VL. Dendrites of the rl nLB cells extend ventrolaterally oriented toward the nDLI and lateral nCLI (asterisk). (Length bar is 250 um.) B) Parasagittal section (rostral is to the right) showing caudal nLB cells and secondary gustatory fibers projecting along the dorsal margin of the 3G to the nSG (arrowheads) labeled after HRP injection in the FL. Note the dendrites of the nLB cells projecting ventrally into nCLI and dorsally into nSG (asterisk). Some of the secondary fibers appear to send collaterals from the nSG ventrally into the nLB. (Length bar is 100 um.)

Figure III.5. Camera lucida drawings of parasagittal (A), horizontal (B), and transverse (C) sections of the posterior tuberculum showing nLB cells and their dendrites filled after HRP injections into the FL. The axon of each cell is indicated by the enclosed white star. D) The location of nLB in a schematic representation of a sagittal section through the brain. (Length bars are 250 um.)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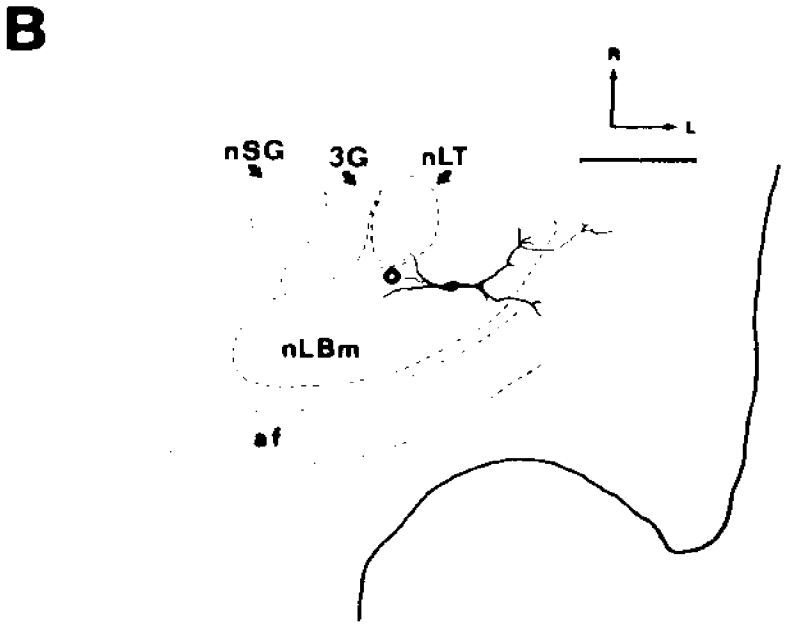
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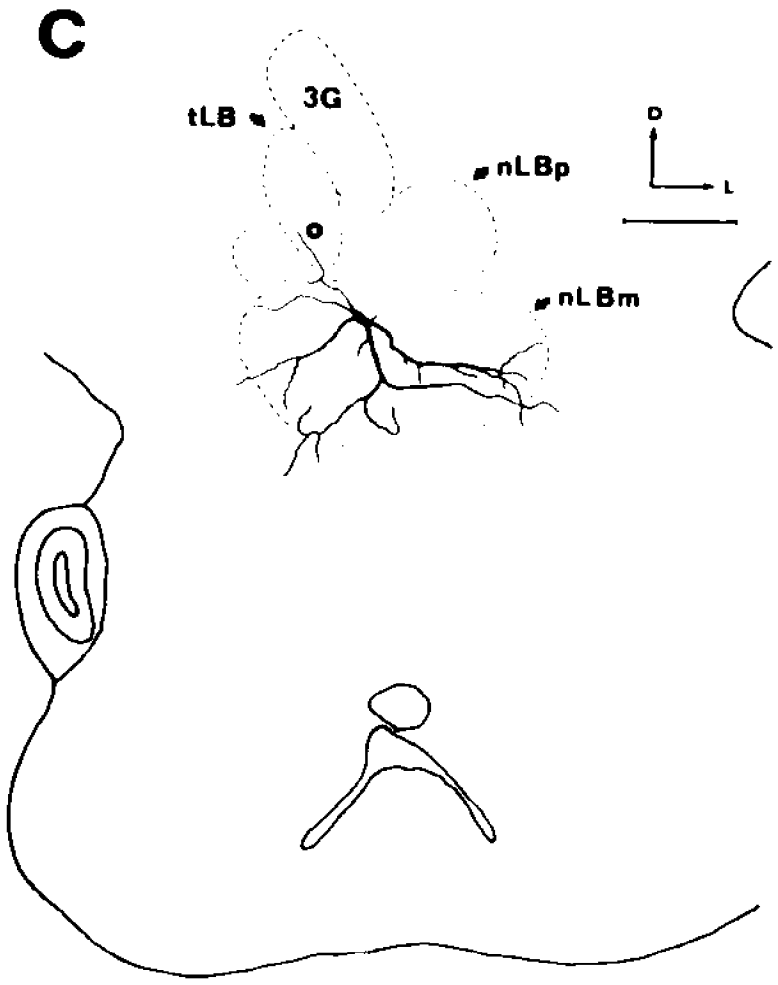

C
tLB
3G
nLBp
nLBm
D
L


D

Injections into the ltfLI of the caudal nucleus centralis labeled two to four adjacent terminal fields (Fig. III.6A). The fibers within each terminal field appeared to be collaterals of 3G fibers which branched off within the rostral portion of nucleus centralis (Fig. III 6B) since this pattern was always accompanied by retrogradely labeled nGS cells (Fig. III.7), and branching 3G fibers were found in the rostral nucleus centralis. Other labeled fibers were diffusely scattered throughout the inferior lobe, terminating in the nucleus lobobulbaris, nLBp, rl nLB, rostral nucleus centralis, nucleus diffusus, nucleus of the lateral recess, lateral thalamic nucleus, and nucleus subglomerulosus (Fig. III.8). Most of these fibers did not extend beyond LI-0 +800 um, but some fibers passed through the nucleus subglomerulosus and lateral thalamic nucleus to terminate in the medial preglomerular nucleus (LI-0 +1200 um). Retrogradely labeled somata were found throughout the rostrocaudal extent of the nucleus centralis (Fig. III.8) and in the nGS. In some cases, the caudal nucleus lobobulbaris cells were lightly filled, with labeled dendrites extending to the injection site and fibers projecting caudally in the tLB to the FL.

Injections into the medial nucleus centralis (LI-0 +200 um to +300 um) produced a similar pattern of projections, but with some notable additions (Fig. III.9). Labeled fibers were found throughout the nucleus centralis, along with projections to the nucleus lobobulbaris, rl nLB, nucleus of the lateral recess, and nucleus subglomerulosus (Fig. III.10). Rostral to the nucleus subglomerulosus were labeled fibers that projected to the medial preglomerular nucleus

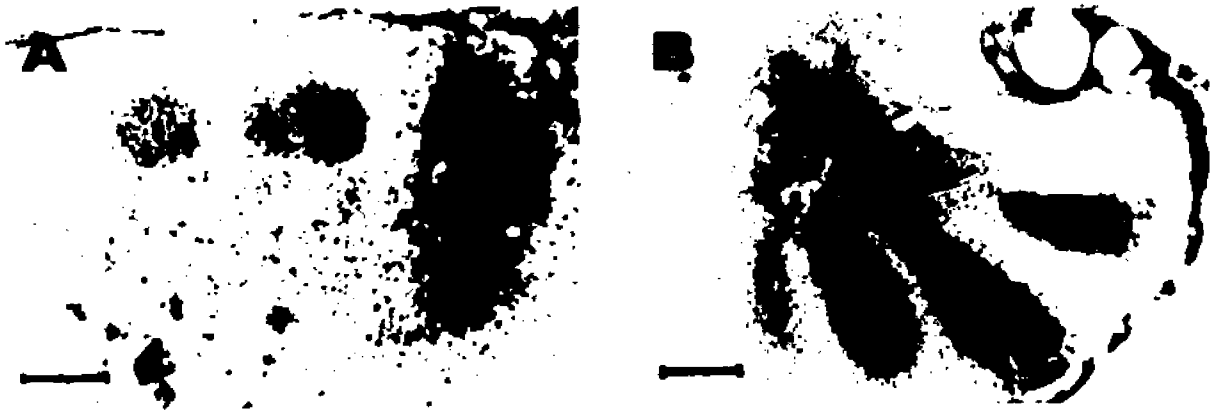


Figure III.6. Sections through the LI after HRP injection into one of the ltfLI. A) Transverse section of the caudal LI (LI-0 -250 um) showing the injection site and two labeled terminal fields in the dorsal nCLI. (Length bar is 100 um.) B) An oblique horizontal section (plane is from dorsomedial at the top of the frame to ventrolateral at the bottom; rostral is to the left) showing four labeled terminal fields after a restricted injection (arrow) into one field (at LI-0 +250 um). There are also numerous retrogradely labeled somata in nCLI. (Length bar is 250 um.)

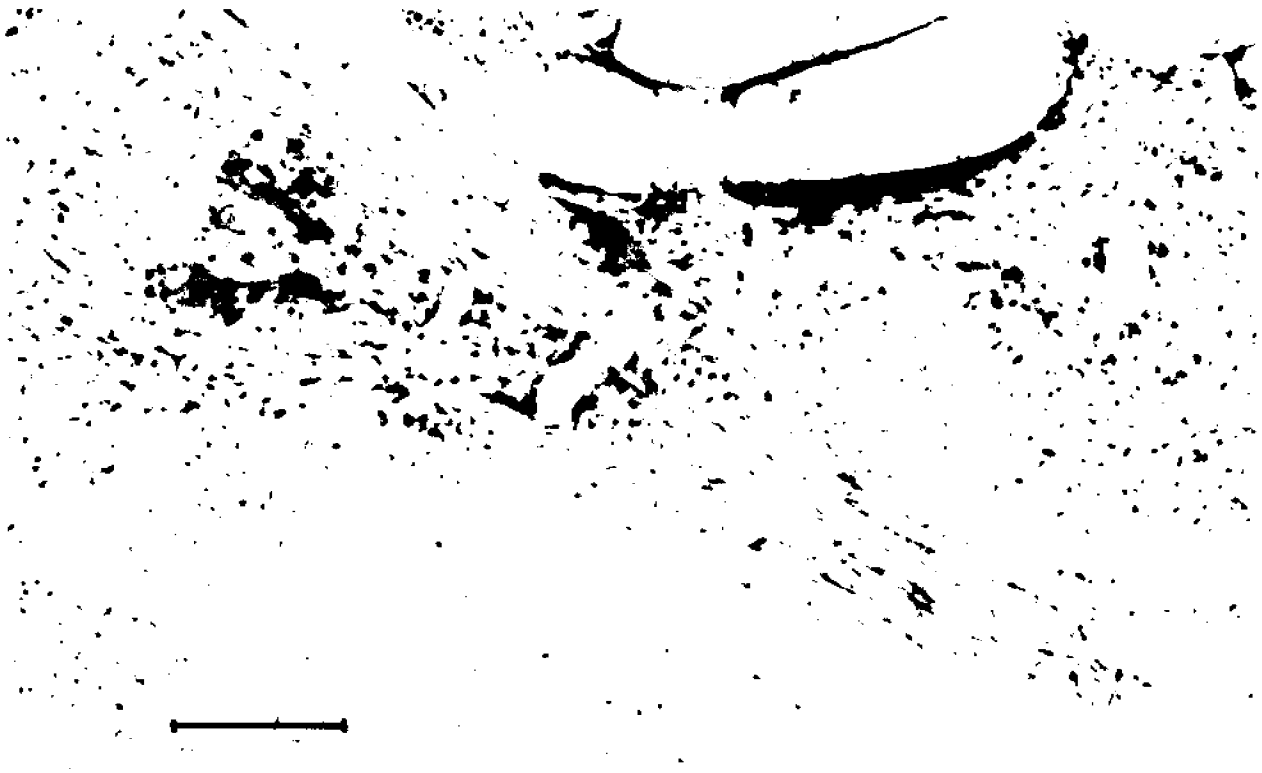
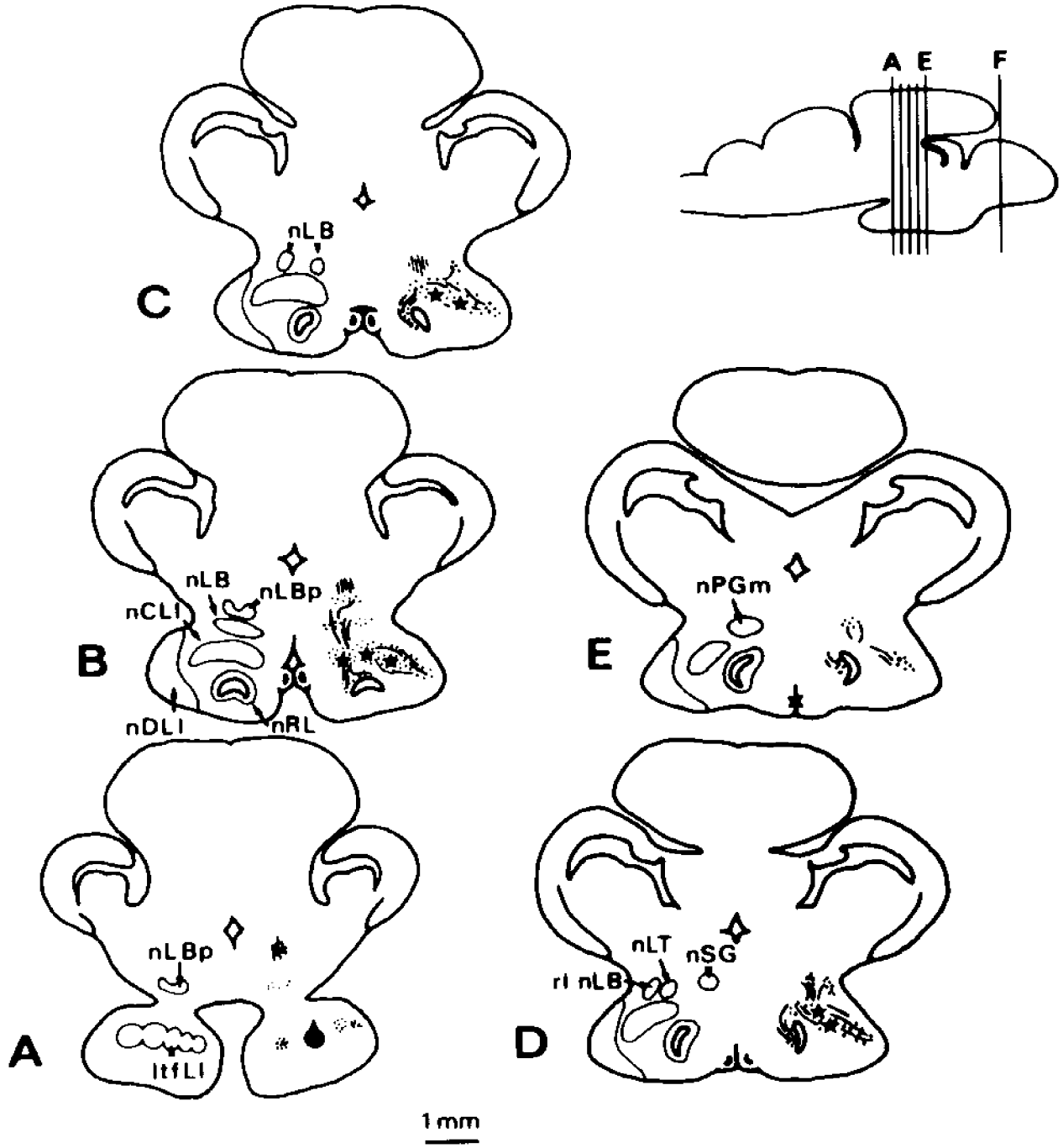


Figure III.7. An oblique horizontal section (from dorsomedial at the top of the frame to ventrolateral at the bottom; rostral is to the right) through the nGS and 3G after HRP injection into the nCLI. Labeled 3G fibers project from ventral nGS cells to cross over the unlabeled tLB as they continue rostrally to the LI. (Length bar is 250 um.)

Figure III.8. Representation of transverse sections through the caudal diencephalon (A-E) summarizing labeled fibers (lines), terminals (dots), and somata (stars) following HRP injections into the caudal portion of nucleus centralis (injection site indicated by enclosed diagonal lines in section A). Nuclear locations are identified on the left side of each section, while the experimental results are depicted on the right side. The first section (A) is at LI-0 and each subsequent section is approximately 200 um rostral to the preceding section. Figure at top right is a sagittal view of the brain showing the approximate location of each section (section F applies to Figs. III.9 and III.12).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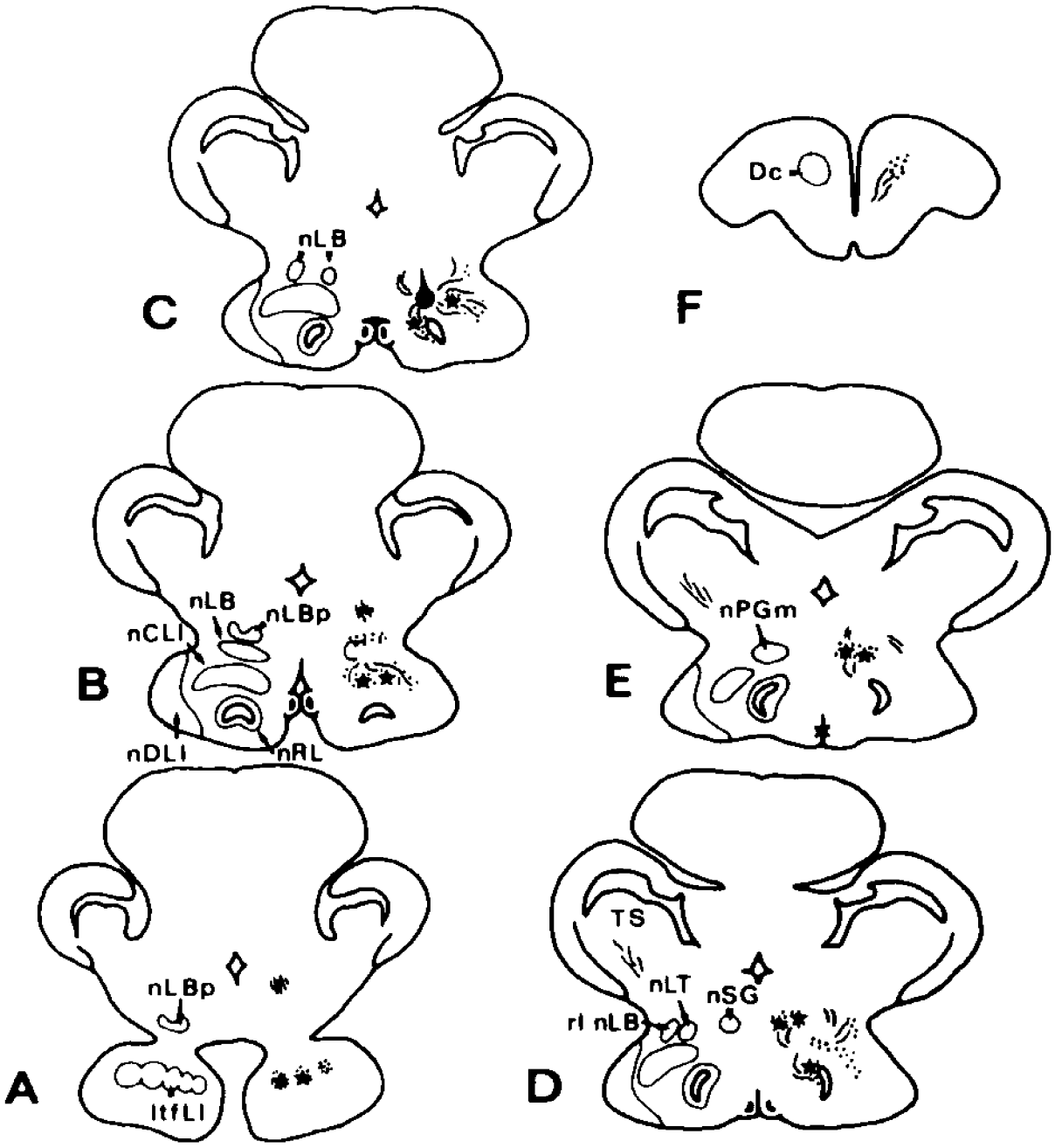
rl nLB
1 mm

Figure III.9. Representation of transverse sections through the caudal diencephalon (A-E) and telencephalon (F) summarizing labeled fibers (lines), terminals (dots), and somata (stars) following HRP injections into the medial portion of nucleus centralis (injection site indicated by enclosed diagonal lines in section C). Nuclear locations are identified on the left side of each section, while the experimental results are depicted on the right side. The first section (A) is at LI-0 and each subsequent section is approximately 200 um rostral to the preceding section (except F; see Fig. III.8 for approximate location of each section).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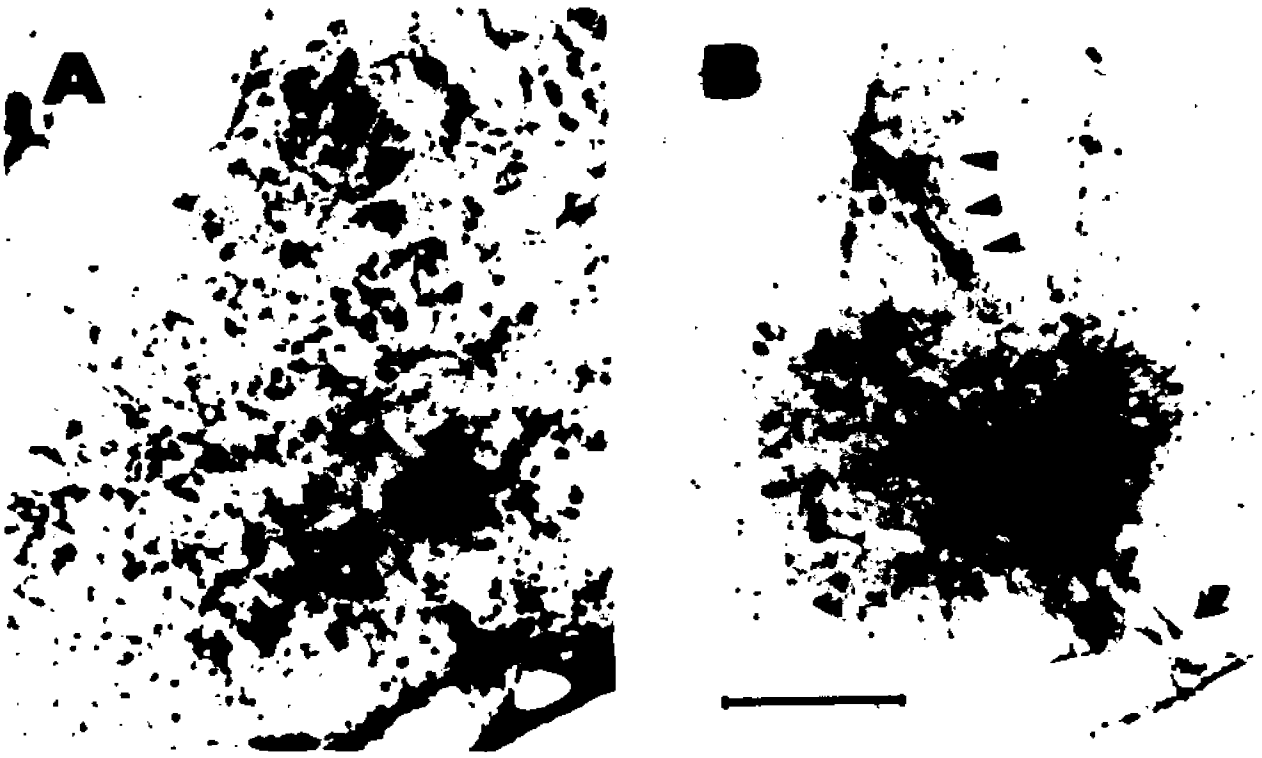


Figure III.10. Two adjacent parasagittal sections through the medial nCLI following an HRP injection (asterisk) approximately 200 um from the lateral recess of the third ventricle. A) Nissl-stained section showing the cells of the nRL (ventral) and nLB (dorsal). B) Unstained section showing labeled cells in the nRL (arrow) and a bundle of fine fibers projecting dorsally into the nLB (arrowheads). (Length bar is 200 um.)

and some that turned laterally at the nPGm to enter the posterior thalamic nucleus (nTP). Other fibers that passed through the nPGm continued rostrally to the horizontal commissure where they crossed to the contralateral side and descended caudally to the deep region of the torus semicircularis (TS) in the contralateral mesencephalic tegmentum. Injections into the medial nucleus centralis also labeled fibers in the medial forebrain bundle (MFB) that terminated in the medial portion of nucleus dorsalis pars centralis (Dc) of the telencephalon. Retrogradely labeled cells were found in the nGS, along with their efferent fibers in the 3G, as described for caudal nucleus centralis injections. When the injection site was within 200-300 um of the lateral recess, fusiform cells of the nucleus of the lateral recess were labeled (Fig. III.10B). These nRL cells were oriented perpendicularly to the ventricular wall so that their processes extended radially into the nucleus centralis. Other labeled somata were found in the nucleus centralis, including the ltfLI (Fig. III.11A), the nucleus lobobulbaris (Fig. III.11B), the nucleus subglomerulosus (Fig. III.11C,D), and the medial preglomerular nucleus. The labeled cells in the ltfLI were medium-sized cells with dendritic processes confined within a single column (Fig. III.11A).

Injections into lateral portions of the nucleus centralis labeled fibers throughout the nCLI and nucleus diffusus, as well as fibers to the nucleus of the lateral recess and nucleus subglomerulosus. The fibers of the 3G were also labeled, but there were fewer collaterals within the ltfLI than after injections into either the caudal or medial nucleus centralis. Other projections

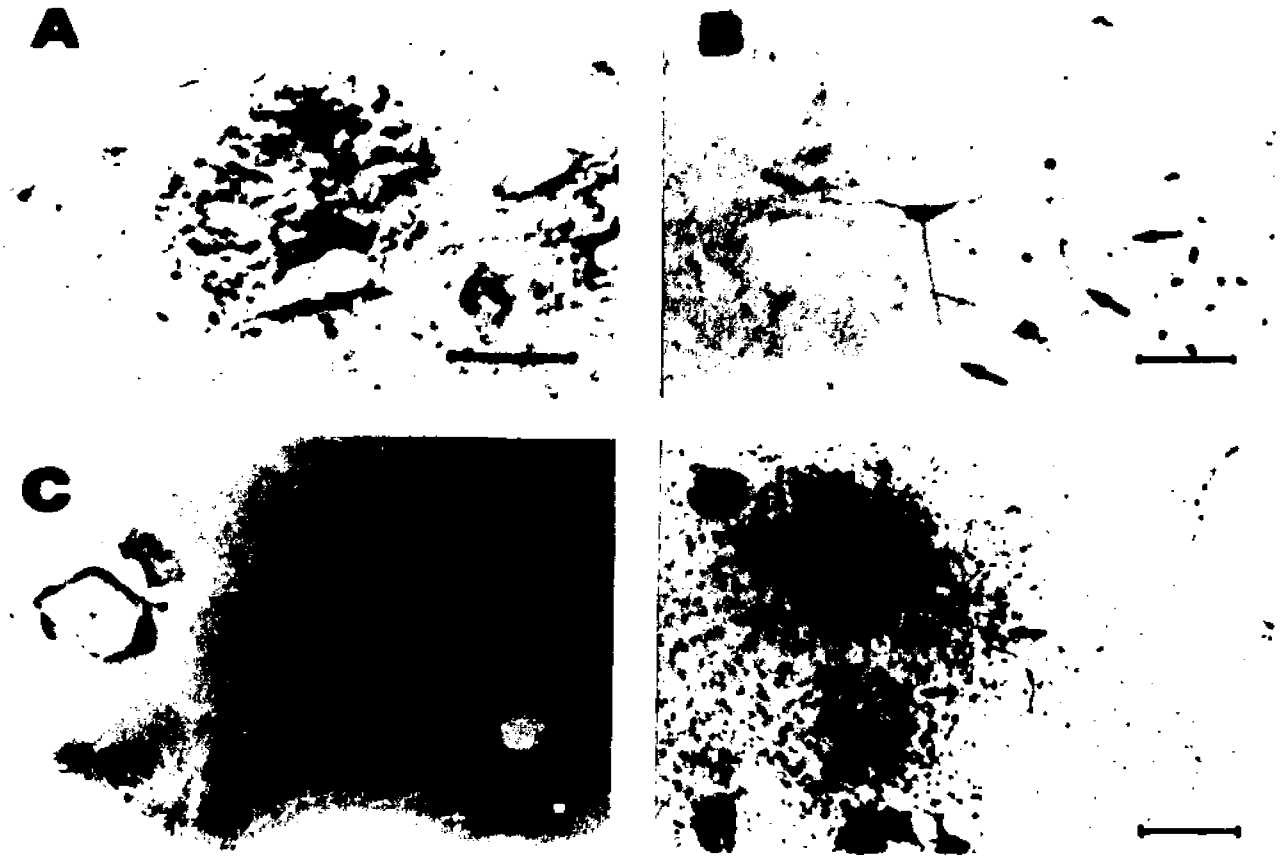
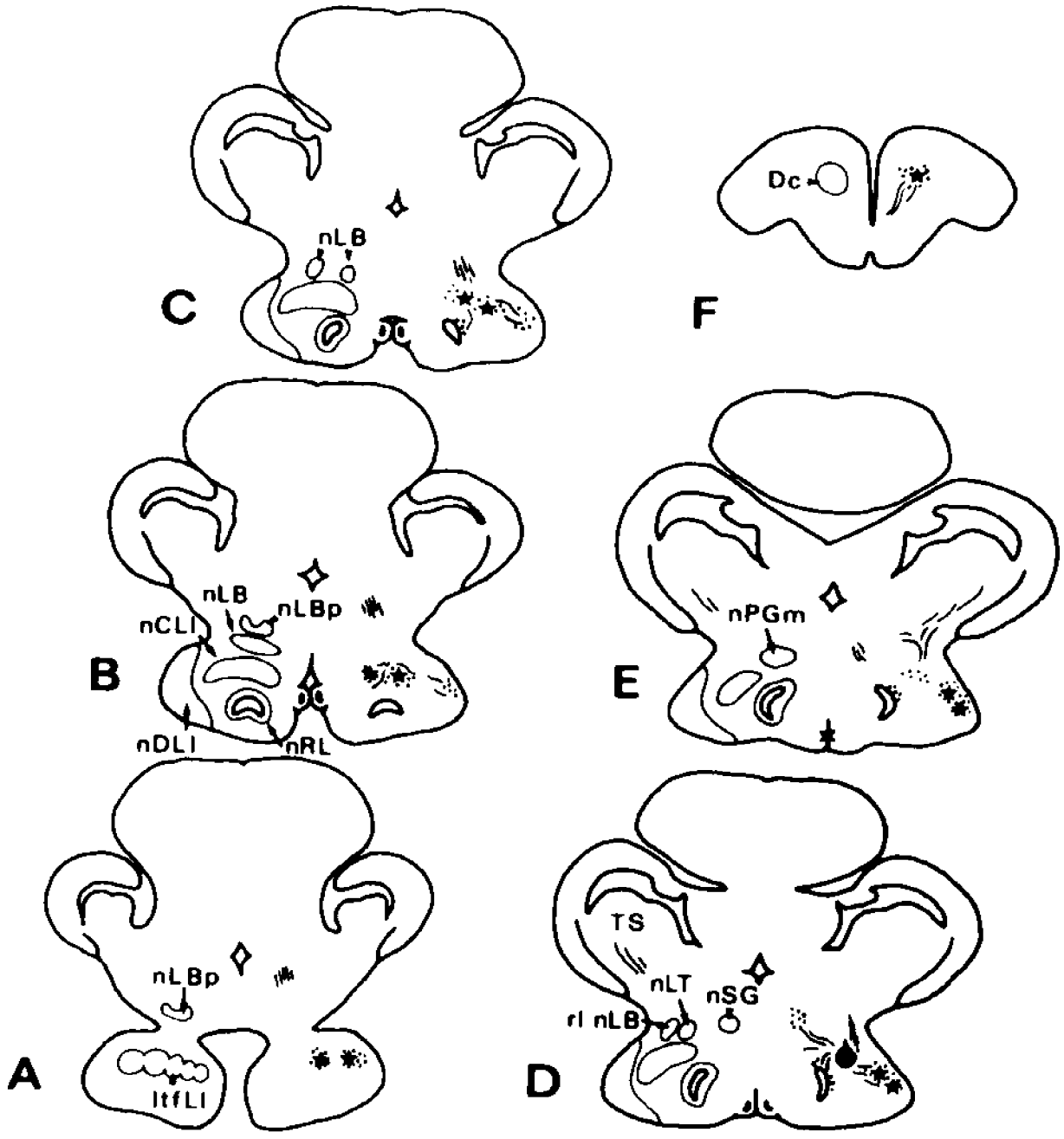


Figure III.11. Labeled somata in the LI after nCLI injections. A) Transverse section of a cell in the caudal nCLI (LI-0 -250 um) with dendrites spanning a single terminal field. B) Transverse section of a caudal nLB cell (LI-0 +200 um) with extensive primary dendrites (arrows) projecting ventrally into the nCLI. C) Parasagittal section (rostral is to the right) through the nSG (arrowheads) showing cells labeled by an injection into the central nCLI. D) Transverse section (LI-0 +750 um) of labeled cells in the nSG (arrowheads) and nCLI (arrows) following an injection into the medial nCLI. (Length bars in A) and B) are 100 um, bars in C) and D) are 250 um.)

found following injections in the lateral nucleus centralis included fibers ascending in the medial forebrain bundle to the area dorsalis pars centralis, and fibers projecting bilaterally to the deep torus semicircularis. Labeled cells were found in the nGS, ltfLI, nucleus centralis, nucleus diffusus (up to LI-0 +1000 um), and Dc (Fig. III.12). Injections near the lateral margin of nucleus centralis, at the medial edge of the lateral nucleus diffusus, labeled cells in the lateral thalamic nucleus with fibers projecting to the nGS, and rl nLB cells with fibers projecting in the tLB back to the VL.

Other injection sites - HRP was injected into two additional regions to confirm some of the suspected projections of the inferior lobe nuclei. In two fish, HRP was injected iontophoretically into the torus semicircularis after obtaining contralateral mechanoresponses from the mouth region. These injections labeled medium-sized cells in the central nucleus centralis (LI-0 +300um to +500 um), whose fibers traveled dorsally through the lateral nCLI and passed between the lateral thalamic nucleus and rl nLB to enter the mesencephalon (not shown). Some of these fibers continued dorsolaterally to the deep torus semicircularis and others terminated in the contralateral torus semicircularis after crossing in the horizontal commissure. Injections into the medial telencephalon of four fish labeled fibers in the medial forebrain bundle which ended throughout the nucleus centralis, in the medial preglomerular nucleus, the nucleus lobobulbaris, and in the caudal portion of the nLBp (Fig. III.13A). Labeled somata were found in the nLBp (LI-0 +50 um) dorsal to the internal arcuate fibers (Fig. III.13A), and throughout the nucleus

Figure III.12. Representation of transverse sections through the caudal diencephalon (A-E) and telencephalon (F) summarizing labeled fibers (lines), terminals (dots), and somata (stars) following HRP injections into the lateral portion of nucleus centralis (injection site indicated by enclosed diagonal lines in section D). Nuclear locations are identified on the left side of each section, while the experimental results are depicted on the right side. The first section (A) is at LI-0 and each subsequent section is approximately 200 um rostral to the preceding section (except F; see Fig. III.8 for approximate location of each section).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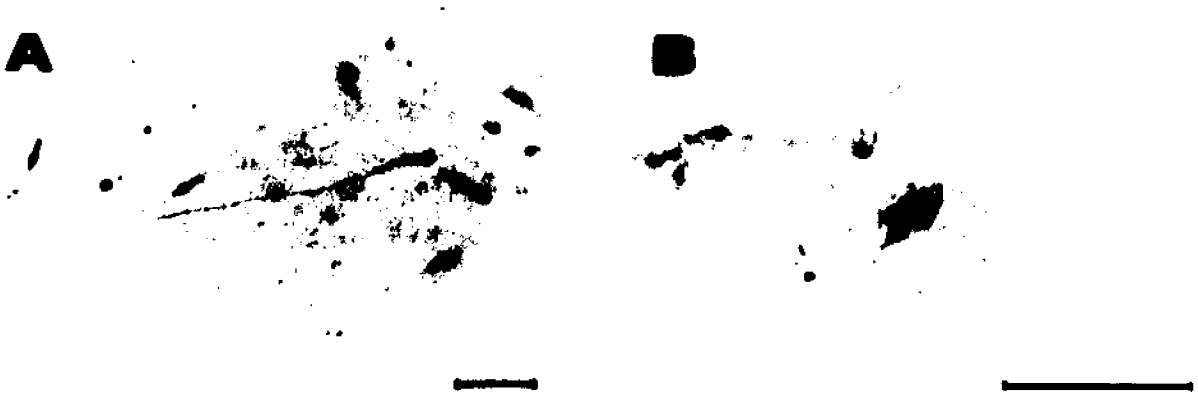


Figure III.13. Retrogradely labeled cells following HRP injections into the medial telencephalon. A) Transverse section through the caudal nLBp (LI-0 +50 um) showing a labeled cell (large arrow) with its axon directed medially toward the medial forebrain bundle, as well as fine descending fibers from the MFB entering the nLBp (small arrows). (Length bar is 100 um.) B) High magnification of a transverse section through the rostral nCLI (LI-0 +700 um) showing HRP granules in an nCLI cell. (Length bar is 50 um.)

centralis (Fig. III.13B). While the nLBp cells were heavily labeled (Fig. III.13A), the nucleus centralis cells typically possessed only a few HRP granules within the soma (Fig. III.13B).

DISCUSSION

The present study provides hodological information regarding the diencephalic recipients of secondary and tertiary gustatory projections in the channel catfish, allowing both an accurate description of higher order interconnections in the silurid taste system and a comparison of these nuclei between different teleost species. Previous anatomical studies of the gustatory system in the teleost brain identified connections of both secondary and tertiary fibers in the diencephalon, but inconsistencies in the nomenclature used for certain diencephalic nuclei and apparent species differences in connectivity have resulted in divergent conclusions.

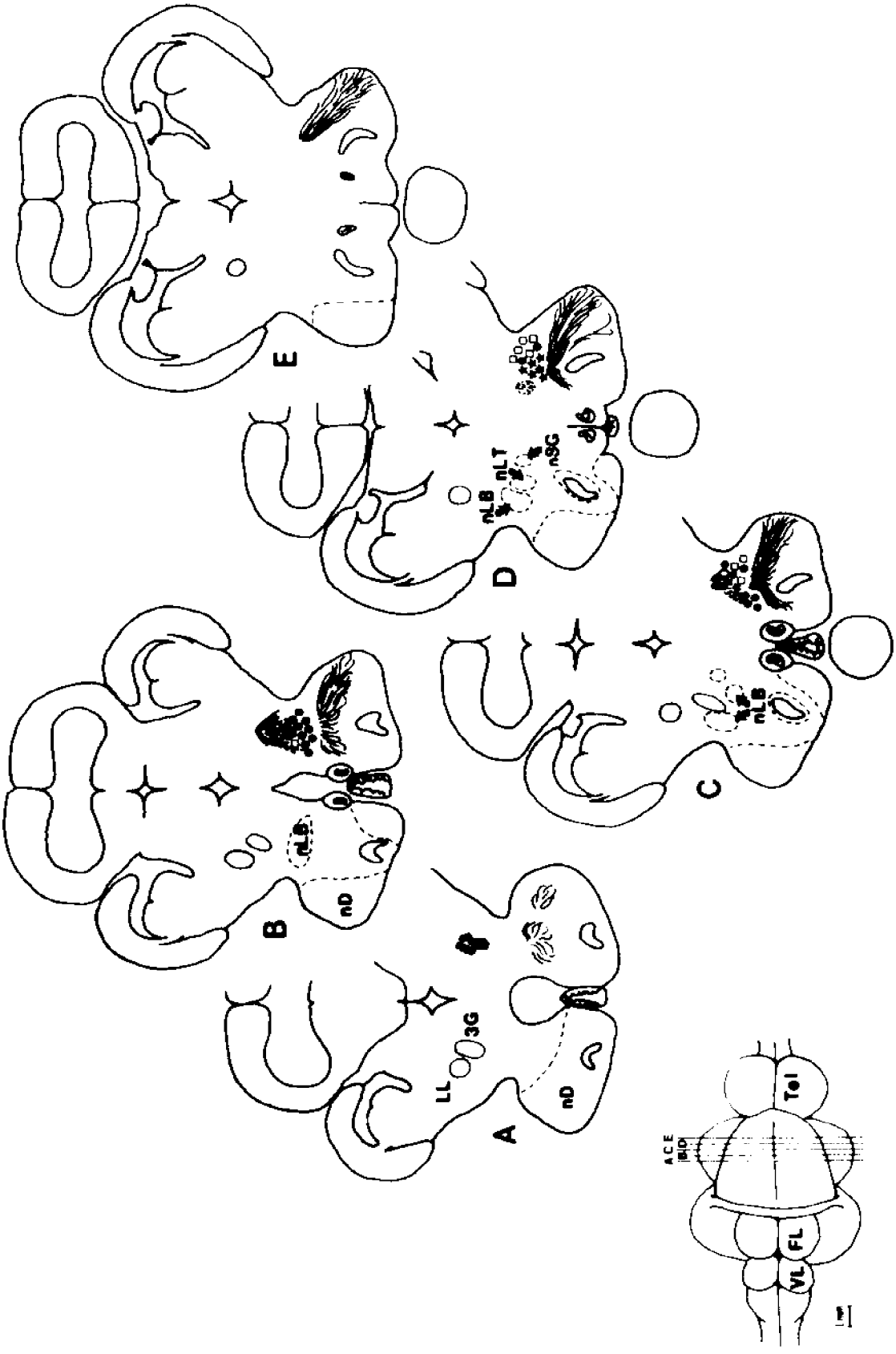
Earlier studies identified nGS efferents entering the caudal diencephalon as the tertiary gustatory tract, but were unable to ascertain the termination sites of these fibers (Herrick, '05; Shanklin, '35; Barnard, '36). Recent experimental studies using degeneration and neuronal labeling techniques identified a number of recipient nuclei for 3G fibers. In the carp, Morita et al. ('80, '83) found 3G projections to the nucleus glomerulosus (nG), nucleus diffusus, and nucleus of the lateral recess. Wullimann ('88) found similar projections in the sunfish, as well as fibers entering the nucleus centralis. Tertiary gustatory fibers in bullhead catfish were found in the posterior thalamic nucleus (nTP), nucleus lobobulbaris, lateral inferior lobe, and ventrolateral nRL (Finger, '83), while in the channel catfish 3G projections were identified in the lateral

thalamic nucleus, nucleus lobobulbaris, and longitudinal terminal fields of the inferior lobe (Kanwal et al., '88).

All of these results are characterized by 3G projections to a nucleus in the posterior tuberculum and to several nuclei in the hypothalamus (see Braford and Northcutt, '83). The posterior tubercular 3G recipient was identified as the nucleus glomerulosus in the carp (Morita et al., '80, '83), but this interpretation seems tenuous since ostariophysines do not possess a recognizable nucleus glomerulosus (Braford and Northcutt, '83). In species with an obvious nucleus glomerulosus, the 3G terminates in a portion of the preglomerular complex (nucleus gustatorious tertius of Wulliman, '88); furthermore, the nucleus glomerulosus is involved in visual pathways and does not receive 3G projections (Sakamoto and Ito, '82). In catfish, the 3G target in the posterior tuberculum was identified as the posterior thalamic nucleus (Finger, '83; Lamb et al., '87) and later as the lateral thalamic nucleus (Kanwal et al., '88; present study). Additional projections were found in the nucleus lobobulbaris (Finger, '83, '88; Kanwal et al., '88; present study).

The nucleus lobobulbaris and lateral thalamic nucleus form a cluster of cells at the margin of the mesencephalic tegmentum and the inferior lobe projecting back to the primary and secondary gustatory nuclei, respectively (Fig. III.14). Three cell groups can be distinguished in the nLB-nLT complex in the catfish by their respective cytoarchitecture and projections. The large multipolar cells of the caudal nucleus lobobulbaris project primarily to the FL (Figs. III.4A, III.5), while the large fusiform cells of the

Figure III.14. Representation of transverse sections through the caudal diencephalon summarizing results of HRP injections into the FL, VL, or nGS. Following FL-injections, labeled fibers (dashed lines) and nLB cells (solid circles) were present in the posterior tuberculum (B-D). VL injections labeled nLB cells (open squares) and their fibers (dotted lines) (B-D). Injections in the nGS labelled 3G efferents (solid lines) in the nCLI (A-D) and lateral nDLI (B-E), and nLT cells (solid stars) (C-D). (Sections are approximately 250 um apart.)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FL
VL

rostrolateral nucleus lobobulbaris (rl nLB) project to the VL (Fig. III.4B). Cells in both the nLB and rl nLB have dendrites that extend into the 3G termination sites in the nucleus centralis and nucleus diffusus, respectively, providing a potentially monosynaptic pathway from the nGS to the FL and VL. In the carp, Morita et al. ('83) found cells in the posterior thalamic nucleus and nucleus diffusus that appear to correspond to the nucleus lobobulbaris of catfish. Cells in the rostrolateral and caudal portions of these nuclei were labeled after HRP injections in the VL, and rostromedial and caudal cells were labelled after FL injections. Unlike the nucleus lobobulbaris of catfish (Finger, '78; Kanwal et al., '88; present study), the nTP and nDLI cells that project to the FL in the carp send efferents bilaterally to the medulla (Morita et al., '83). Luiten and van der Pers ('77) reported ipsilateral projections from the nucleus of the lateral recess, nucleus diffusus, and nucleus preglomerulosus to the motor nuclei of the facial and trigeminal nerves in the carp. It is unclear whether there are differential projections from the inferior lobe to the sensory and motor nuclei of the carp or if the nucleus preglomerulosus of Luiten and van der Pers ('77) is equivalent to the posterior thalamic nucleus of Morita et al. ('83).

The present results confirm that the nucleus lobobulbaris parvicellularis (nLBp) of Kanwal et al. ('88) is involved in ascending gustatory pathways, since it receives fibers from the FL, nGS, and caudal nucleus centralis, and nLBp neurons project to the medial telencephalon (Table III.2). Further evidence is provided by a recent electrophysiological study reporting gustatory responses of nLBp

Table III.2. Summary of HRP-labeled gustatory connections in the inferior lobe of the channel catfish. (All connections are ipsilateral unless otherwise noted; see text for abbreviations.)

Nucleus		Efferent fibers	Retrogradely labeled cells
nCLI:	ltfLI-	nCLI, nDLI, nLB, rl nLB nLBp, nLT, nRL, nPGm	nCLI, nGS
	rostromedia.-	ltfLI, nCLI, nLB, rl nLB nRL, nSG, nPGm, nTP, Dc contralateral TS	ltfLI, nCLI, nGS nRL, nSG, nPGm
	rostrolateral-	ltfLI, nCLI, nDLI nRL, nSG, Dc bilateral TS	ltfLI, nCLI, nDLI nGS, Dc
nLB:	caudal-	VL, FL, nVIIm, nVm	
	rostrolateral-	VL	
	nLBp-	Dc	
nLT:		nGS	nGS
Others:			
	VL-		nLB, rl nLB
	FL-	nLB, nLBp, nSG	nLB
	nGS-	ltfLI, nCLI, nDLI, nLB rl nLB, nLBp	nLT
	TS-	bilateral nCLI, nDLI, nLB	nCLI
	medial Tel.-	nCLI, nLB, nLBp, nPGm	nCLI, nLBp

neurons in the channel catfish to amino acids applied to the oral cavity and extraoral body surface (see Chapter IV). The parvicellular cell group of the nucleus lobobulbaris can be distinguished from the caudal and rostrolateral portions of the nucleus by several anatomical features. Even though the cells of the nLBp and nLB are interspersed where the 3G enters the nucleus lobobulbaris, at caudal levels the nLBp is a distinct group of cells located adjacent to the 3G in the tegmentum. Also, the cell morphology of nLBp cells differs from that of the nLB and rl nLB neurons, and nLBp efferents project to the telencephalon (Kanwal et al., '88; present study) while nLB and rl nLB cells project to the medulla (Finger, '78; Morita and Finger, '85; Kanwal et al., '88; present study). It is not known if the nLBp is unique to catfish, since a similar cell group has not been identified in any other group of fishes. Comparative studies of the nuclei associated with the medial forebrain bundle in other teleosts could possibly identify homologues of this telencephalopetal cell group.

The lateral thalamic nucleus of Kanwal et al. ('88) might also be unique to silurids, since cells corresponding to the medium-sized cells of the nLT that project back to the nGS in catfish (Lamb et al., '87; Kanwal et al., '88; present study) were not identified in either the carp (Morita et al., '83) or the sunfish (Wullimann, '88). However, Morita et al. ('83) did identify cells in and nearby the nucleus of the lateral recess of the hypothalamus that projected to the nGS in the carp. Based on cytoarchitecture and topology, Striedter ('90a) suggested that the nucleus lobobulbaris and lateral thalamic nucleus of catfish are homologous to the lateral thalamic

nucleus of cyprinids (Braford and Northcutt, '83). Connectional evidence from the present study, however, indicates that the lateral thalamic nucleus was misnamed in catfish, since the nLT of cyprinids (Grover and Sharma, '81; Luiten, '81) and centrarchids (Striedter and Northcutt, '89) projects to the optic tectum and is involved in visual pathways, and there is no similar visual nucleus in the catfish (Striedter, '90b). The catfish nucleus lobobulbaris and lateral thalamic nucleus appear to be feedback pathways for the reflexive transmission of gustatory information to the medulla and nGS, respectively (Lamb et al., '87; see below). Because of the highly derived nature of the posterior tuberculum in catfish, studies of the gustatory pathways of non-ostariophysine teleosts are needed to identify possible homologies for gustatory nuclei in silurids.

Hypothalamic nuclei receiving 3G projections include the nucleus centralis, nucleus diffusus, and nucleus of the lateral recess in all teleosts examined (Morita et al., '80, '83; Finger, '83; Kanwal et al., '88; Wullimann, '88; present study). The present study indicates that these nuclei form a gustatory center in the hypothalamus of teleosts. The majority of 3G efferents terminate in the nucleus centralis, from the antero-posterior level of the lateral thalamic nucleus back to the ltfLI in the caudal inferior lobe, and in the lateral nucleus diffusus (Fig. III.14). The nucleus centralis is characterized by a network of connections with other gustatory-related nuclei in the posterior tuberculum and hypothalamus, as well as extrinsic connections with the mesencephalic tegmentum and the

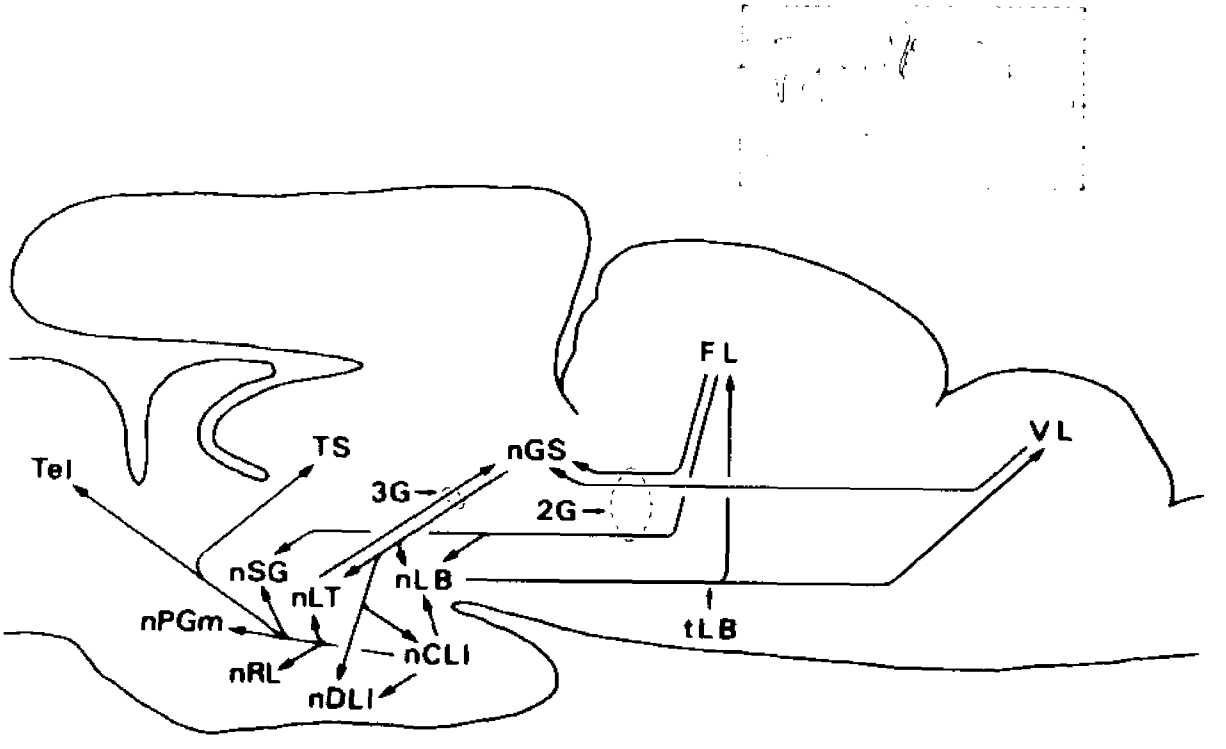

Figure III.15. Schematic diagram of gustatory connections of the posterior tuberculum and caudal hypothalamus. Not shown are the reciprocal projections from the nDLI, nSG, nPGm, and Dc (Tel) to the nCLI, and the connections of nLBp (see Table III.2).

telencephalon (Fig. III.15; Table III.2). Within the inferior lobe, the nucleus centralis has reciprocal connections throughout different regions of the nucleus (ltfLI, rostromedial nCLI, and rostrolateral nCLI), and with the nucleus of the lateral recess and the nucleus subglomerulosus. The nucleus subglomerulosus receives 2G projections from the facial lobe, and appears to be equivalent to the posterior thalamic nucleus descibed previously in the bullhead catfish (Finger, '78). The nucleus of the lateral recess is a periventricular nucleus surrounding the lateral recess of the third ventricle, with cell processes extending out into the central portion of the inferior lobe (Demski et al., '75; Evan et al., '76). The nRL of the stickleback (Ekstrom et al., '86) and goldfish (Hornby and Piekut, '90) receives noradrenergic projections from the isthmus through the lateral tegmental tract, and contributes to the dopaminergic innervation of the inferior lobe (Hornby et al., '87). The nRL also contains a number of other neuroactive substances, based on immunoreactivity of nRL cells in the molly to antisera for corticotrophin-releasing factor, enkephalin, somatostatin, thyrotrophin-releasing hormone, cholecystokinin, and substance P (Batten et al., '90). Many of these peptides have been implicated in mechanisms of feeding and related autonomic processes in the mammalian ventral forebrain (see Riche et al., '90).

Functional studies of feeding behavior in teleosts identified the nucleus of the lateral recess, nucleus subglomerulosus, nucleus centralis, and nucleus diffusus as constituents of a hypothalamic feeding center. Electrical stimulation of these nuclei produced

complete feeding responses in bluegill (Demski and Knigge, '71), tilapia (Demski, '73), and goldfish (Savage and Roberts, '75). The evoked responses typically included searching the tank, snapping at food, and snapping up gravel, although the presence of food or gravel was not required for the response (see Demski, '83). Savage and Roberts ('75) suggested that stimulation near the nucleus subglomerulosus and nucleus of the lateral recess of goldfish induced motivational shifts responsible for the feeding responses, since the resultant behavioral pattern was complete and it varied with competing sensory cues. Lesioning experiments support the presence of a hypothalamic feeding center in teleosts. Damage to the lateral hypothalamic region of goldfish, including the nucleus of the lateral recess, nucleus centralis, and nucleus diffusus, produced pronounced aphagia and hypophagia (Roberts and Savage, '78). The deficit affected both operant feeding and manually presented food, and was significantly less pronounced when the lesion was located in other regions of the hypothalamus.

Electrophysiological recording from neurons in this region also support the notion of a hypothalamic feeding center in teleosts. Cells near the nucleus of the lateral recess of the goldfish responded to electrical stimulation of the VL and olfactory tract and to food extract applied to the oral cavity (Demski, '81). Preliminary electrophysiological results from this region in the channel catfish also identified cells responsive to amino acids and mechanical stimulation applied to the extraoral surface (Kanwal et al., '88).

Unfortunately, neither of these studies identified the specific location of the recording electrode; however, a recent study of the diencephalon of the channel catfish identified gustatory responses from neurons in the nucleus centralis, as well as in the nLB, rl nLB, nLBp, and lateral thalamic nucleus of the posterior tuberculum (see Chapter IV).

Other notable connections of the nucleus centralis include the medial preglomerular nucleus, the torus semicircularis, and the medial portion of the area dorsalis pars centralis of the telencephalon (Dc). Because of possible 3G projections to the nPGm (Finger, '88; Striedter, '90a), this nucleus in catfish was previously suggested by Striedter ('90a) as the homologue of the nucleus glomerulosus of the carp (Morita et al., '80, '83) and the tertiary gustatory nucleus of the sunfish (Wullimann, '88). The present results indicate that 3G projections to the posterior tuberculum of the channel catfish are restricted to the lateral thalamic nucleus and nucleus lobobulbaris, and that the nPGm receives only indirect gustatory projections through the nucleus centralis.

The torus semicircularis of the catfish, an important mesencephalic relay center for acousticolateral sensory systems, is composed of three distinct regions associated with the auditory system, and the mechanosensory and electrosensory lateral line systems, respectively (Knudsen, '77; Tong and Finger, '83; Finger and Tong, '84). Although the specific termination sites of nucleus centralis efferents in the TS were not identified in the present study, the projection from the nucleus centralis to the TS suggests an

integration of exteroceptive sensory input, including both gustatory and acousticolateral systems.

Previous studies identified reciprocal connections of the nucleus centralis and nucleus diffusus with the telencephalon in the channel catfish (Striedter, '90b) and the goldfish (Airhart, '87). The present results confirm this connection and identify the medial portion of Dc as at least one of the telencephalic nuclei receiving nucleus centralis efferents. An anatomical and electrophysiological investigation of the gustatory connections of the forebrain in the channel catfish recorded taste activity from the region of the telencephalon including Dc and the ventral portion of area dorsalis pars medialis, and identified the nLBp as the source for this ascending gustatory information (Kanwal et al., '88). Although both nLBp and nucleus centralis project to the telencephalon (present study), it seems more likely, based on the relative density of gustatory projections to the nLBp and nucleus centralis, respectively, that the nCLI is the primary source of telencephalopetal gustatory input (Striedter, '90b). A recent electrophysiological examination of the nuclei of the caudal inferior lobe in the channel catfish found taste responsive neurons in the nLBp and nucleus centralis (Chapter IV); however, the relative roles of these two nuclei are unknown and functional conclusions regarding their projections to the telencephalon are still premature.

The gustatory pathways in the hindbrain of widely divergent vertebrate groups are strikingly similar, providing easy comparisons

between taxa (Herrick, '44; Norgren and Leonard, '73). However, major differences in the construction of the diencephalon between the two most widely studied groups, teleosts and mammals, limit those comparisons to the primary and secondary gustatory nuclei. Tertiary gustatory fibers in the rat project from the parabrachial nucleus to several forebrain regions, including the parvicellular portion of the ventral posteromedial thalamic nucleus, central nucleus of the amygdala, bed nucleus of the stria terminalis, and the lateral hypothalamus (Norgren and Leonard, '73; Norgren, 76; Saper and Leowy, '80). These regions, aside from the VPMpc, send fibers back to the parabrachial nucleus (Fulwiler and Saper, '84) and the nucleus of the solitary tract (van der Kooy et al., '84). Reciprocal connections were also found between the parabrachial nucleus and the insular cortex of the rat (Saper, '82).

Similar to the ventral forebrain centers of the mammalian gustatory system (see Norgren, '85), the caudal portion of the inferior lobe of the channel catfish receives tertiary gustatory fibers and sends efferents back to both the primary and secondary gustatory nuclei (see Fig. III.15). Furthermore, 3G projections in both teleosts (Batten et al., '90) and mammals (Shimada et al., '85; Schwaber et al., '88; Yasui et al., '89) display immunoreactivity to calcitonin gene-related peptide. The cells of origin for this projection were identified as the nucleus lateralis valvulae in the molly (Batten et al., '90); however, a recent study of the general visceral pathways in catfish identified an isthmic secondary visceral nucleus that was immunoreactive to calcitonin gene-related peptide and

projected to the inferior lobe (Finger and Kanwal, submitted). This association of general visceral and special visceral (gustatory) nuclei in the isthmic region of catfish, along with the tertiary projections from these nuclei to the diencephalon, is similar to ascending visceral and gustatory projections from the parabrachial nucleus of the rat (Fulwiler and Saper, '84).

The gustatory connections in the diencephalon of the catfish are simpler (present study) than the densely interconnected radial arrangement of gustatory pathways in the mammalian ventral forebrain (Norgren, '85; Luiten et al., '87), and might represent a functional equivalent to the mammalian lateral hypothalamus (Finger, '88). Previous studies in catfish suggested the presence of a lemniscal gustatory pathway to the telencephalon, similar to the mammalian pathway from the parabrachial nucleus through the dorsal thalamus to the insular cortex (Finger, '83; Kanwal et al., '88). Many different nuclei in the diencephalon of teleosts have been proposed as homologues of sensory relay nuclei in the thalamus of tetrapods (see Braford and Northcutt, '83); however, diencephalic gustatory connections in the channel catfish are restricted to the posterior tuberculum and caudal hypothalamus (present study), and do not include nuclei in the region considered homologous to the dorsal thalamus of tetrapods (Braford and Northcutt, '83; Striedter, '90b).

Supporting evidence for the similarity between the caudal portion of the inferior lobe of teleosts and the lateral hypothalamus of mammals comes from previous studies utilizing electrical

stimulation or lesioning in these regions (see above for summary of this work in teleosts). Electrical stimulation in the lateral hypothalamus of rabbits (Schwartzbaum, '88) and rats (Coons et al., '65; Roberts, '80) produced eating and various components of feeding behavior, including gnawing, chewing, and licking. Similar to electrical stimulation of the goldfish inferior lobe (Savage and Roberts, '75), stimulation of the rat lateral hypothalamus produced a motivational response that resembled the effects of normal hunger (Coons et al., '65). This elicitation of feeding behaviors by lateral hypothalamic stimulation in rats was associated with increased glucose metabolism in the lateral tegmental pathway from the lateral hypothalamus to the parabrachial nucleus (Roberts, '80), as well as a selective enhancement of gustatory responsiveness of neurons in the nucleus of the solitary tract (Matsuo et al., '84). Unfortunately, there is no information regarding the specific mechanism underlying the feeding responses of teleosts following inferior lobe stimulation. Neuronal damage induced by iontophoretic injections of kainic acid into the lateral hypothalamus of the rat produced transient aphagia and prolonged hypophagia (Grossman and Grossman, '82), similar to the effects of damage to the lateral and central portions of the goldfish inferior lobe (Roberts and Savage, '78). More information on the functional relationships of the constituent cell groups of the gustatory pathways in the inferior lobe of fishes such as the catfish is required, however, before these similarities in behavioral responses can be used to identify homologous structures and pathways between teleosts and mammals.

The present results identify a discrete set of connections between the gustatory centers in the hindbrain and proposed gustatory nuclei in the caudal inferior lobe of the channel catfish. The diencephalic nuclei that receive secondary and tertiary gustatory projections include cell groups that send efferents back to the hindbrain centers and other cell groups that project to multisensory mesencephalic regions and to the telencephalon. A connectional basis is provided for the comparison of these nuclei between different teleosts, as well as a comparison of ascending gustatory pathways in different vertebrates. Further research in the gustatory pathways of other anamniotes will help determine whether the gustatory systems of different vertebrates involve modifications of a similar design, or if the gustatory connections identified in teleosts and mammals are constituents of a spectrum of different pathways evolved for gustation (see Northcutt, '81).

Chapter IV

Taste and Tactile Responsiveness in the Posterior Diencephalon

INTRODUCTION

Gustatory pathways in all vertebrates examined have exhibited common patterns of organization throughout the brainstem, including similar primary medullary nuclei associated with afferent fibers of the facial (VII), glossopharyngeal (IX), and vagal nerves (X), and important gustatory relays in the metencephalon and diencephalon (catfish: Fig. IV.1). Early anatomical studies identified tertiary gustatory projections from the isthmic region of the metencephalon to the posterior diencephalon in teleosts (Herrick, '05) and amphibians (Herrick, '44). More recently, modern neuroanatomical techniques were used to specify these tertiary projections in different teleost species. Efferents from the superior secondary gustatory nucleus in carp (Morita et al., '80, '83), catfish (Finger, '83; Lamb et al., '87; Kanwal et al., '88; Chapter III), and sunfish (Wullimann, '89) terminate in the caudal portion of the hypothalamic inferior lobe. Using recent comparative studies of the diencephalon of teleosts (Braford and Northcutt, '83; Striedter, '90a) for nuclear definitions, the recipient nuclei of tertiary gustatory projections in catfish were identified as the nucleus lobobulbaris of the posterior tuberculum and the hypothalamic nucleus centralis and nucleus diffusus of the inferior lobe (Chapter III). Ascending secondary gustatory fibers from the medullary facial lobe also project to this region (Finger, '78; Kanwal et al., '88), terminating in the nucleus lobobulbaris and nucleus subglomerulosus (Chapter III). Neurons in the nucleus lobobulbaris project back to the facial and vagal lobes of the medulla

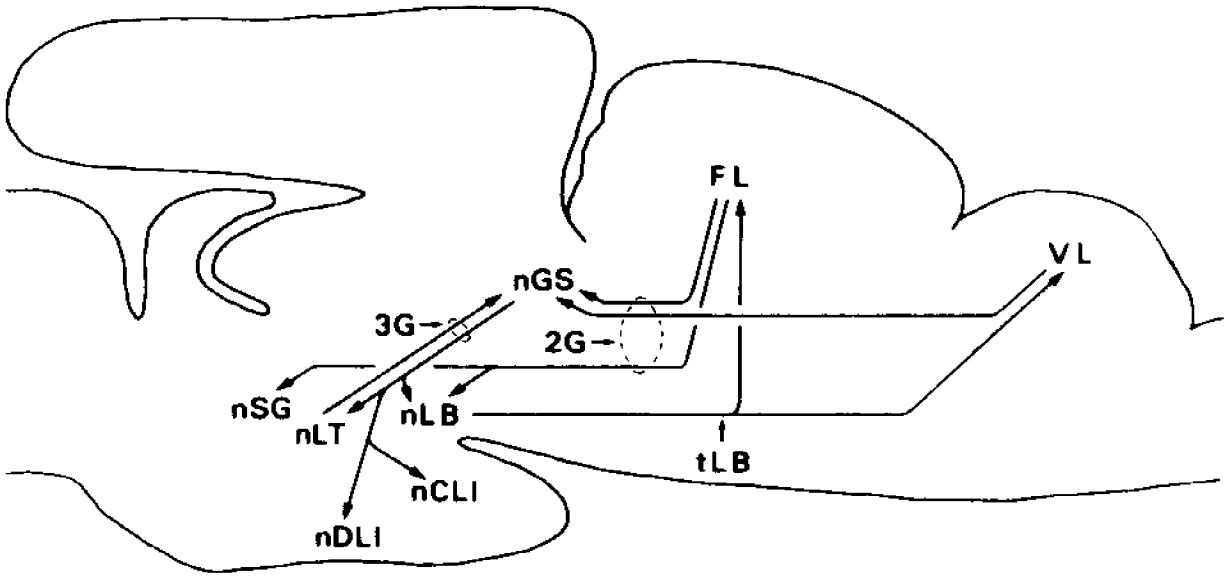

Figure IV.1. Schematic diagram of the connections of the gustatory nuclei in the medulla (VL and FL) and pons (nGS) with nuclei in the posterior tuberculum (nLB, nLT, and nSG) and hypothalamus (nCLI and nDLI) of the channel catfish. (2G - secondary gustatory tract, 3G - tertiary gustatory tract, FL - facial lobe, nCLI - nucleus centralis of the inferior lobe, nDLI - nucleus diffusus of the inferior lobe, nGS - superior secondary gustatory nucleus, nLB - nucleus lobobulbaris, nLT - lateral thalamic nucleus, nSG - nucleus subglomerulosus, tLB - lobobulbar tract, VL - vagal lobe)

(Finger, '78; Lamb et al., '87; Kanwal et al., '88; Chapter III) and, through the medial forebrain bundle, to the telencephalon (Kanwal et al., '88; Chapter III).

Similar tertiary projections from the pontine parabrachial nucleus to the thalamus and lateral hypothalamus were identified in mammals, as well as additional tertiary projections to other forebrain nuclei (Norgren and Leonard, '73; Saper and Loewy, '80; Block and Schwartzbaum, '83; Fulwiler and Saper, '84). Lateral hypothalamic efferents, in part, terminate in the primary (nucleus of the solitary tract: Saper et al., '79; van der Kooy et al., '84) and secondary (parabrachial nucleus: Fulwiler and Saper, '84) gustatory nuclei. Numerous electrophysiological studies have characterized the gustatory nature of the parabrachial nucleus (Norgren and Pfaffmann, '75; Scott and Perrotto, '80; Yamamoto et al., '80; Ogawa et al., '82; Schwartzbaum, '83; Hermann and Rogers, '85; Nishijo and Norgren, '90; Halsell and Frank, '91), and correlated gustatory activity of parabrachial neurons with projections to the thalamus (Hayama et al., '87; Ogawa et al., '87) and ventral forebrain (Block and Schwartzbaum, '83). Additional studies involving tertiary gustatory projections to the mammalian diencephalon recorded gustatory activity in the thalamus (Benjamin, '63; Scott and Erickson, '71; Ninomiya and Funakoshi, '82; Nomura and Ogawa, '85; Pritchard et al., '89) and lateral hypothalamus (Norgren, '70; Schwartzbaum, '88).

Unlike the extensive characterization of central gustatory activity in mammals, few electrophysiological studies have recorded taste responses in teleosts. Initial electrophysiological studies of

neurons within the facial lobe (Marui, '77), vagal lobe (Vasilevskaya and Polyakova, '78, '80), and superior secondary gustatory nucleus (Marui, '81) of carp tested gustatory stimuli patterned after the four primary tastes of mammals - salty, sweet, sour, and bitter - thus stimuli generally included sodium salts, sugars, acids, and quinine, respectively. The majority of electrophysiological studies of peripheral gustatory nerves in teleosts, however, have identified amino acids as the more potent and relevant taste stimuli for fishes (see Caprio, '84, '88). More recently, taste responses to amino acids were detected in the vagal (Kanwal and Caprio, '88) and facial (Marui and Caprio, '82; Marui et al., '88; Hayama and Caprio, '89) lobes of catfish, but those studies were primarily concerned with characterizing the topographical organization of the medullary gustatory centers and they tested more extensively for tactile responsiveness. The only study to report dose-dependent responses of single units to several amino acids was a recent study of the superior secondary gustatory nucleus in the channel catfish (Chapter II). The only previous studies of gustatory activity in the diencephalon of teleosts identified responses to amino acids in the channel catfish (Kanwal et al., '88) and to food extracts in goldfish (Demski, '81); however, neither of these studies determined the cell groups responsible for the taste responses.

The present study is an electrophysiological investigation of the nuclei in the caudal inferior lobe of the channel catfish, Ictalurus punctatus, that were identified by previous anatomical

studies as recipients of ascending gustatory projections (see Chapter III). Single units were tested for responsiveness to both taste and tactile stimuli to determine response profiles to different tastants as well as receptive field organization and basic response characteristics. Each recording site was marked and later identified to compare responsiveness between different nuclei.

MATERIALS and METHODS

Twenty-five channel catfish, weighing from 10 to 65 g, were immobilized with an intramuscular injection of Flaxedil (gallamine triethiodide, 0.5 mg/kg) and secured in a Plexiglass container. The gills and oral cavity were perfused with aerated, charcoal-filtered city tap water (artesian well water), and supplemental doses of Flaxedil were administered as required. The dorsal surface of the head was anaesthetized by topical application of 3% tetracaine. The parietal bone was removed dorsal to the cerebellum and the mesenchymal tissue was withdrawn to expose the cerebellum and the rostral portion of the facial lobes. Electrical activity within the inferior lobe was recorded extracellularly by glass microelectrodes (2-6 um tip diameter; 1-5 Mohm impedance) filled with 2% pontamine sky blue in 0.5 M sodium acetate. Using features of the dorsal surface of the cerebellum as landmarks, the electrode was driven vertically through the cerebellum in a grid of tracks 250 um apart.

Searching for units in the inferior lobe consisted of systematically testing portions of the oropharyngeal cavity and extraoral body surface with mechanical and chemical stimuli while the electrode was in the vicinity of the gustatory nuclei. Usually, between 3.5 and 4.0 mm below the surface of the cerebellum, the contralateral mechanosensory receptive fields present in the mesencephalic tegmentum would abruptly cease, and ipsilateral mechanoresponses would indicate the electrode was located in the dorsal portion of the inferior lobe. Tactile stimulation was produced

by stroking different portions of the surface of the fish with a thin glass rod to identify "glide-type" units (Biedenbach, '73). Chemical stimulation consisted of three different solutions: (1) a bovine liver extract (10 g/l; gravity filtered), (2) a mixture of amino acids (L-ala, L-arg, L-pro, and D-ala, each at 0.1 mM), and (3) a mixture of nucleic acid derivatives (adenine, adenosine, AMP, and ATP, each at 10 mM) as search solutions. Individual amino acids and nucleic acid derivatives were tested once chemoresponsive activity was confirmed. Chemical stimuli were delivered as 0.5 ml aliquots into a constant water flow (12 ml/min) directed at portions of the body surface or oropharyngeal cavity. The constant water flow allowed for adaptation of the initial mechanical response prior to chemical stimulation, leaving only a small pressure pulse caused by switching from background to stimulus. Amplified unit activity was recorded on magnetic tape and subsequently analyzed with a dual window discriminator (BAK, DIS-1) or by computer (Brainwave) to isolate single unit responses.

Electrode placement was verified histologically by iontophoretic marking with pontamine sky blue (10 uAmps of cathodal DC current for 30 minutes). After recording and dye marking, the animal was anaesthetized with tricaine methane sulfonate (MS-222, 100 mg/L) and perfused intracardially with teleost Ringer's solution followed by 4% glutaraldehyde in 0.1 M phosphate buffer (7.2 pH). The brain was removed and embedded in egg yolk, post-fixed for 4 hours, and placed in a sucrose buffer solution for 12-24 hours (Morita and Finger, '85).

The brain was sectioned transversely at 33-50 um on a freezing microtome and the sections were counter-stained with neutral red (Bures et al., '83). Recorded responses were grouped by nuclei according to previous anatomical analyses of the diencephalic cell groups of the channel catfish (Striedter, '90a; Chapter III) and of other actinopterygian fishes (Braford and Northcutt, '83).

RESULTS

A total of 63 single unit and 133 multiunit preparations were recorded from 106 electrode tracks through the posterior inferior lobe in 25 specimens. Of the single units isolated, 73% (46) responded only to tactile stimulation, and 27% (17) responded to both tactile and taste stimuli. There were no units that responded only to taste stimulation. Both single and multiunit responses were recorded in the nucleus centralis of the inferior lobe (nCLI), nucleus lobobulbaris (nLB), lateral thalamic nucleus (nLT), and nucleus subglomerulosus (nSG; Fig. IV.2). Only multiunit responses, however, were obtained from the nucleus diffusus of the inferior lobe (nDLI) and the longitudinal terminal fields (ltfLI) in the caudal portion of nucleus centralis. The following descriptions are organized by diencephalic region, covering first the gustatory nuclei in the hypothalamus (nCLI and nDLI) and then those in the posterior tuberculum (nLB, nLT, and nSG). Only single units were included in the analysis of tactile and taste responsiveness (Table IV.1); however, multiunit responses are included in the following nuclear descriptions for those cell groups in which no single units were recorded.

Nucleus centralis - Ten single units were identified in the nucleus centralis. Most of these units responded with phasic increases in activity to tactile stimulation of the whole body (Table IV.1). Eight of the recorded units had no spontaneous activity and neither of the remaining two were suppressed by stimulation. Nine units had receptive fields (RFs) covering the whole extraoral body

Figure IV.2. Transverse sections through the inferior lobe of six different specimens showing iontophoretically injected pontamine sky blue deposits subsequent to the recording of single unit activity. A) Electrode location of a unit having extraoral taste and tactile RFs (Fig. IV.3) in the medial nucleus centralis (arrow). Additional dye deposits fom other recording sites are present in the parvicellular nucleus lobobulbaris (top) and lateral nucleus centralis (right). B) Electrode location of a unit having ipsilateral extraoral taste and tactile RFs (Fig. IV.4) from the lateral magnocellular cells of the caudal portion of nucleus lobobulbaris (arrow). C) Electrode location of a unit having ipsilateral oral and extraoral taste and tactile RFs (Fig. IV.5) in the medial magnocellular portion of nucleus lobobulbaris (arrow). D) Electrode location of a unit with complex facilitation/suppression responses to tactile stimulation of the ipsilateral head and facilitation to stimulation of the oral cavity and flanks (Fig. IV.6) in the rostrolateral nucleus lobobulbaris (arrow). An additional dye deposit from another recording site is also visible more medially in the nucleus lobobulbaris. E) Electrode location of a unit having ipsilateral extraoral tactile RFs (Fig. IV.7) in the rostral portion of the lateral thalamic nucleus (arrow). F) Electrode location of a unit having oral and extraoral taste and tactile RFs (Fig. IV.8) in the rostral portion of the lateral thalamic nucleus (arrow). An additional deposit from another recording site is visible in the rostral portion of nucleus centralis (left).

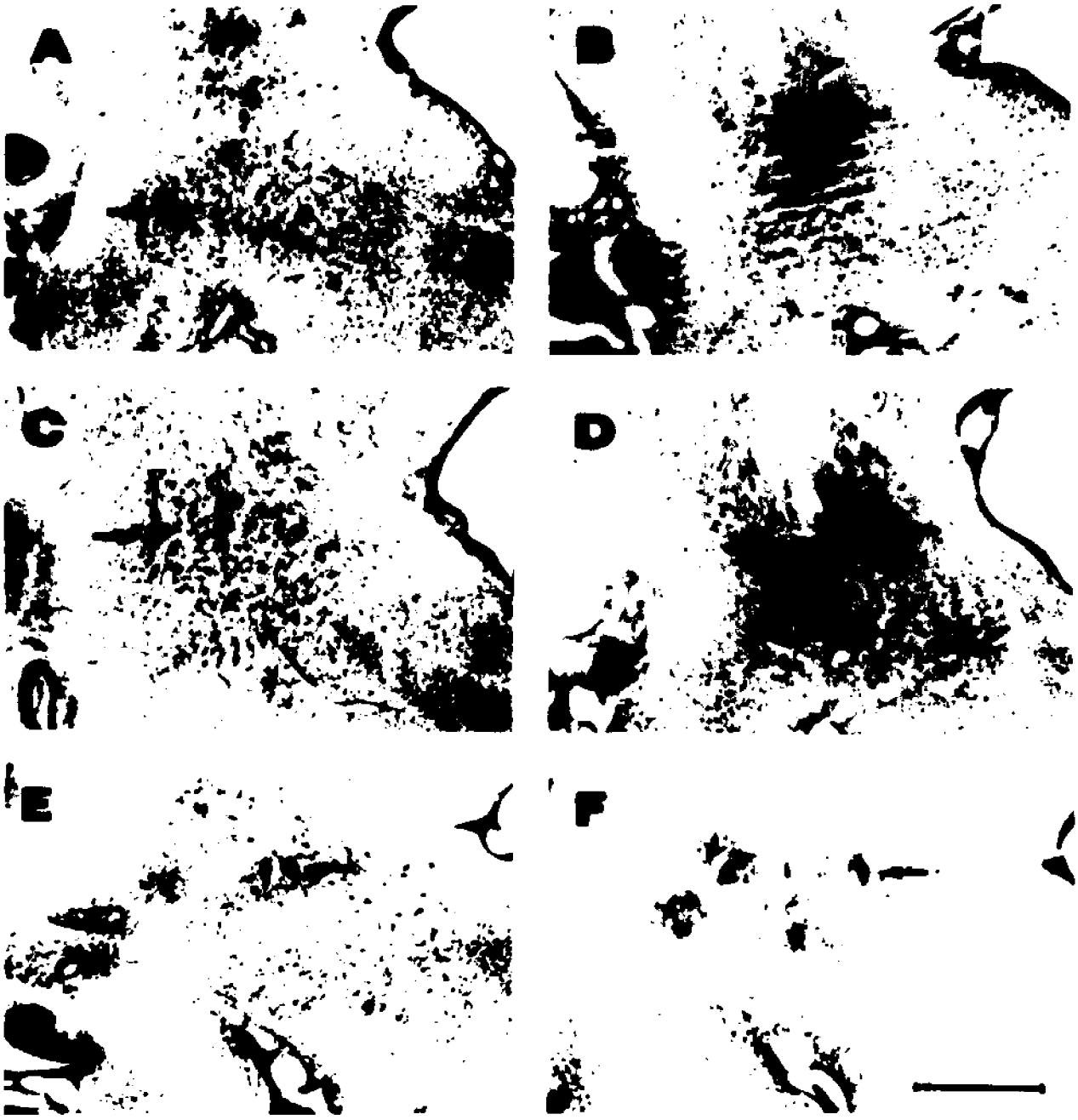
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Table IV.1. Response characteristics of neurons in the gustatory nuclei in the caudal inferior lobe of the channel catfish (mouth - lips and barbels; head - extraoral surface from lips to opercula; body - extraoral surface from lips to caudal fin; oral - surfaces within oropharyngeal cavity; see text for abbreviations).

Nucleus:	nCLI	nLB	r1 nLB	nLBp	nLT	nSG	Total
No. units	10	20	13	13	6	1	63
Spontaneous Activity (spikes/sec):							
Mean	1.35	1.65	3.57	4.46	5.12	0.1	2.88
Range	0-12	0-17.4	0-7.1	0-16.9	0.5-15	NA	0-17.4
Standard error	1.52	0.86	0.57	1.45	2.02	NA	
No. units w/ no spont. act.	8(80%)	12(60%)	1(8%)	4(31%	0	0	25(40%)
Receptive Fields:							
Ipsilateral	6(60%)	17(85%)	6(46%)	4(31%)	5(83%)	1	39(62%)
Bilateral	4(40%)	3(15%)	7(54%)	9(69%)	1(17%)	0	24(38%)
Whole body	9(90%)	15(75%)	11(85%)	6(46%)	4(67%)	1	45(71%)
Head only	1(10%)	1(5%)	2(15%)	4(31%)	1(17%)	0	9(14%)
Mouth only	0	4(20%)	0	2(15%)	1(17%)	0	7(11%)
Extraoral only	7(70%)	17(85%)	2(15%)	2(15%)	5(83%)	1	34(54%)
Oral only	0	0	0	1(18%)	0	0	1(2%)
Both	3(30%)	3(15%)	11(85%)	10(77%)	1(17%)	0	28(44%)
Suppressed	0	0	5(38%)	0	1(17%)	0	6(10%)
+ then - (*)	0	0	3(23%)	3(23%)	0	0	6(10%)
Chemosensory	2(20%)	8(40%)	3(23%)	3(23%)	1(17%)	0	17(27%)

(*) Response consisted of a brief period of phasic facilitation followed by suppression.

surface while one was restricted to the head. Three units responded to stimulation of the oropharyngeal cavity as well as the extraoral surface. Two units also responded to taste stimulation, and one of these units remained functionally isolated to allow extensive testing. This unit was obtained from the medial portion of the nucleus centralis (Fig. IV.2A) and responded to tactile and taste stimulation of the ipsilateral head (Fig. IV.3). Responses were obtained to L-arginine, L-proline, L-alanine, and D-alanine, each applied at 1×10^{-2} M, respectively. A mixture of these amino acids was stimulatory at 4×10^{-3} M (each component at 10^{-3} M), but lower concentrations of each individual amino acid applied separately were not stimulatory to this unit (not shown). The nucleic acid mixture was also stimulatory at 10^{-2} M, but of the individual components only adenine and ATP were effective stimuli at 10^{-2} M (Fig. IV.3).

While single units were not isolated in the caudal portion of nucleus centralis (the longitudinal terminal fields), twenty multiunit recordings were identified from this region (not shown). All of these multiunit preparations responded to tactile stimulation of the extraoral body surface, and fifteen had RFs restricted to the ipsilateral mouth or head.

Nucleus diffusus - No single units were recorded from the nucleus diffusus; however, ten multiunit recordings from this cell group all displayed tactile RFs including both the ipsilateral oropharyngeal cavity and extraoral body surface (not shown). Taste responses were not obtained from this nucleus.

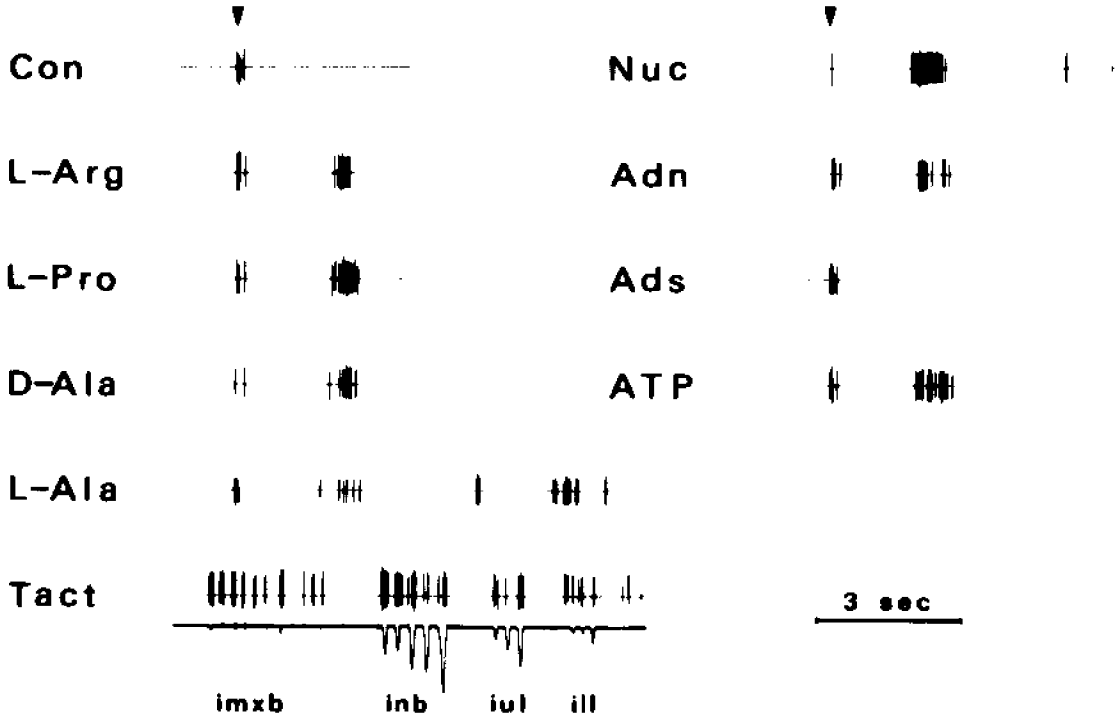
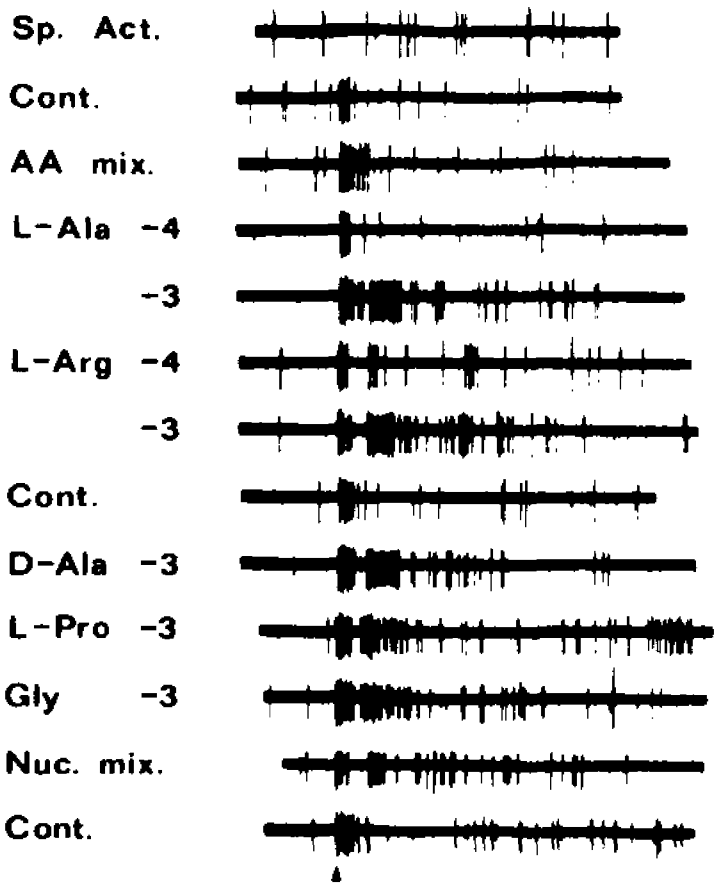


Figure IV.3. Bimodal extraoral responses of a unit from the medial portion of nucleus centralis (Fig. IV.2A). All taste stimuli were applied at 10^{-2} M except the nucleotide mixture, which included adenine, adenosine, and ATP each at 10^{-2} M. The arrowheads indicate the pressure pulse associated with the onset of taste stimulation. The line beneath the tactile responses indicates deflection of stimulating probe. (Adn - adenine, Ads - adenosine, ATP - adenosine triphosphate, Con - well-water control, D-Ala - D-alanine, ill - ipsilateral lower lip, imxb - ipsilateral maxillary barbel, inb - ipsilateral nasal barbel, iul - ipsilateral upper lip, L-Ala - L-alanine, L-Arg - L-arginine, L-Pro - L-proline, Nuc - mixture of nucleic acid derivatives, Tact - tactile responses)

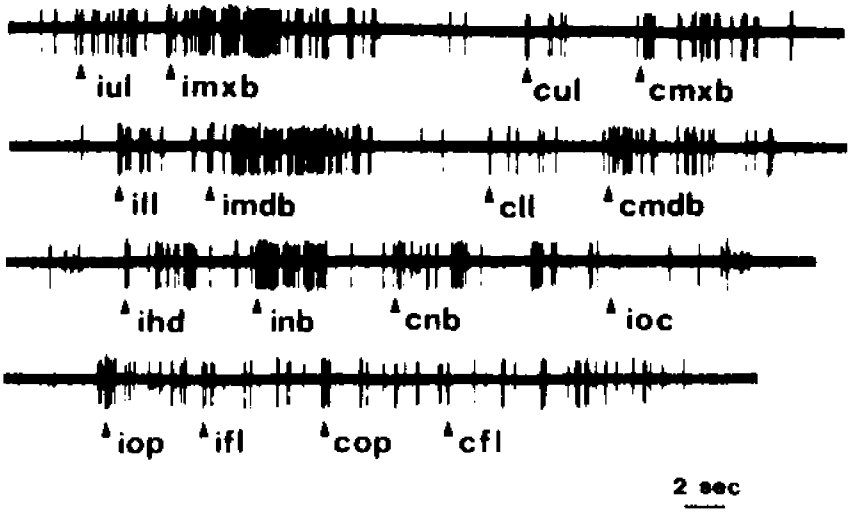
Nucleus lobobulbaris - The nucleus lobobulbaris is divisible into three cell groups based on location, cellular morphology, and connectional patterns (Chapter III). The response characteristics of units within each cell group displayed patterns that differed between the cell groups (Table IV.1). Of the twenty single units functionally isolated in the caudal magnocellular portion of the nucleus lobobulbaris (nLB), twelve units lacked spontaneous activity, and all of the units were facilitated by tactile stimulation. Seventeen of the twenty units had RFs on the extraoral surface only and three included both oral and extraoral surfaces. Fifteen of the extraoral RFs included the body surface from the mouth to the tail, one covered the head, and four were limited to the lips and barbels. Seventeen units had only ipsilateral RFs, while three had similar fields on both sides of the body. Eight units in the caudal nucleus lobobulbaris also responded to taste stimulation of the tactile RFs. One bimodal unit from the lateral portion of the caudal lobobulbar cell group (Fig. IV.2B) responded to several amino acids applied to the ipsilateral maxillary barbel (Fig. IV.4A) and to tactile stimulation of the ipsilateral head (Fig. IV.4B). L-alanine, L-arginine, L-proline, D-alanine, and glycine were effective at 10^{-3} M, and L-arginine alone produced a slight response at 10^{-4} M. This unit also responded to the mixture of nucleic acid derivatives (each component at 10^{-3} M). Another unit from the medial portion of the caudal cell group (Fig. IV.2C) responded to tactile stimulation of the ipsilateral mouth and oral cavity and to taste stimulation of the ipsilateral maxillary barbel with amino acids at micromolar concentrations (Fig.

Figure IV.4. Bimodal responses of a unit from the caudal portion of nucleus lobobulbaris (Fig. IV.2B). A) Taste responses to several amino acids and the nucleotide mixture applied to the ipsilateral maxillary barbel. B) Tactile responses to stimulation of the ipsilateral head. (AA mix - search mixture of amino acids at 10^{-4} M each, cfl - contralateral flank, cll - contralateral lower lip, cmdb - contralateral mandibular barbels, cmxb - contralateral maxillary barbel, cnb - contralateral nasal barbel, Cont - well-water control, cop - contralateral operculum, cul - contralateral upper lip, D-Ala - D-alanine, Gly - glycine, ifl - ipsilateral flank, ihd - ipsilateral head, ill - ipsilateral lower lip, imdb - ipsilateral mandibular barbels, imxb - ipsilateral maxillary barbel, inb - ipsilateral nasal barbel, ioc - ipsilateral oropharyngeal cavity, iop - ipsilateral operculum, iul - ipsilateral upper lip, L-Ala - L-alanine, L-Arg - L-arginine, L-Pro - L-proline, Nuc mix - mixture of nucleic acid derivatives at 10^{-3} M each, Sp Act - spontaneous activity; arrowheads indicate onset of stimulation; numbers indicate exponent of the log molar concentration of each stimulus)

A


Sp. Act.
Cont.
AA mix.
L-Ala -4
-3
L-Arg -4
-3
Cont.
D-Ala -3
L-Pro -3
Gly -3
Nuc. mix.
Cont.

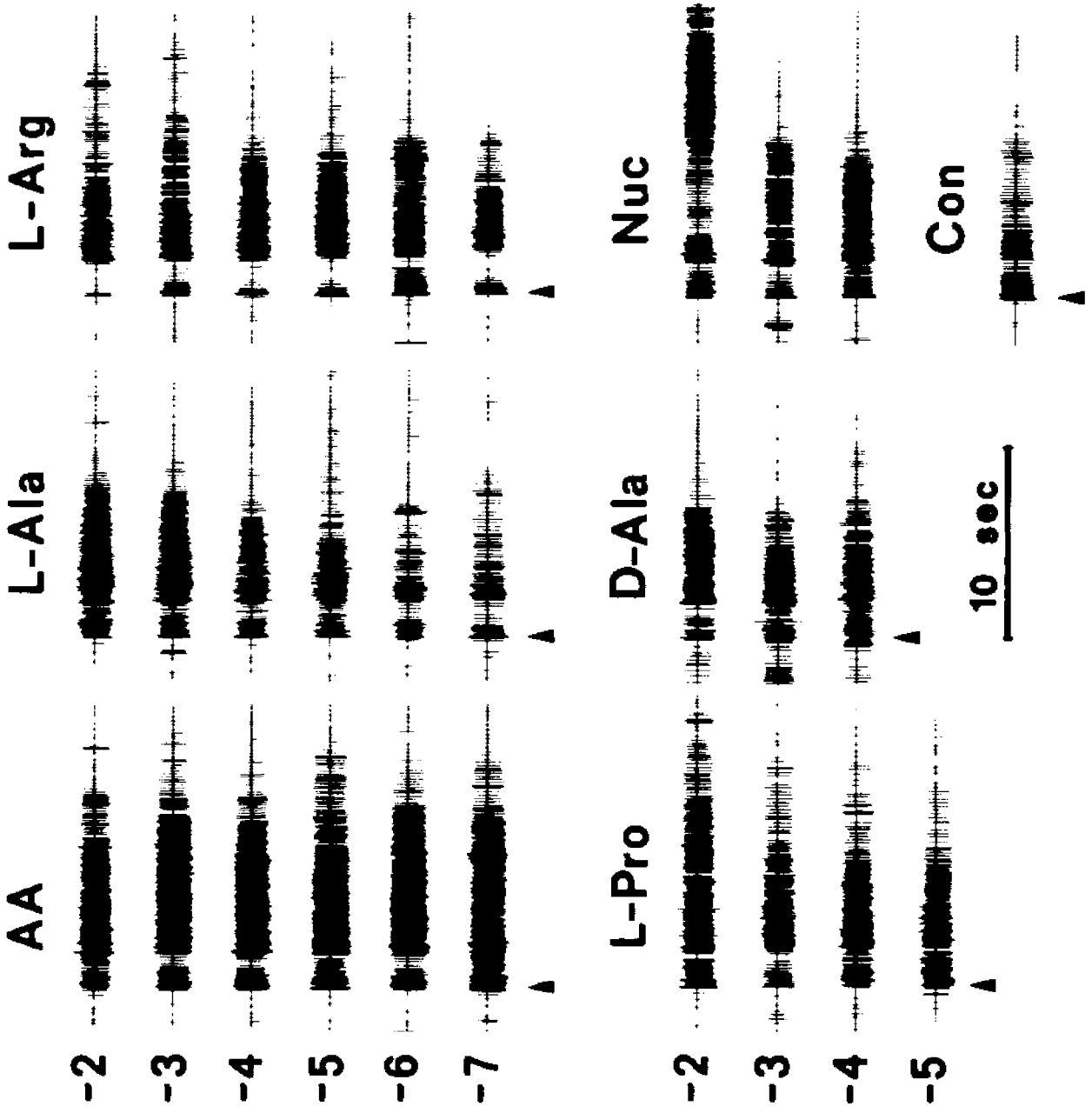
B


iul
imxb
cul
cmxb
ill
imdb
cll
cmdb
ihd
inb
cnb
ioc
iop
ifl
cop
cfl
2 sec

IV.5). The response frequencies of this unit increased with increasing stimulus concentrations above 4 x 10^{-7} M for the amino acid mixture (each component at 10^{-7} M), 10^{-6} M for L-alanine and L-arginine, and 10^{-4} M for L-proline, D-alanine, and the mixture of nucleic acid derivatives (Fig. IV.5). Other units from the caudal nucleus lobobulbaris responded to taste stimuli (amino acid mixture, L-alanine, or L-arginine) at 10^{-4} M or 10^{-3} M, but were not maintained long enough for complete testing.

The other group of magnocellular nucleus lobobulbaris cells forms the rostrolateral extension of the nucleus (rl nLB). Thirteen single units were functionally isolated in this portion of the nucleus lobobulbaris, and their resting and response characteristics differed from those of the caudal cell group (Table IV.1). The spontaneous rates for units in the rostrolateral region were typically higher than those in the caudal region, and only one rostrolateral unit lacked spontaneous activity. Eleven of the units had tactile RFs that included both oral and extraoral surfaces, while two units displayed extraoral RFs only. Neurons in this region also displayed more complex response patterns than those from the caudal nucleus lobobulbaris (Table IV.1). Five units were suppressed by tactile stimulation of their RFs and three responded with phasic facilitation followed by a brief period of suppression. An example of the latter response pattern is shown by the smaller unit in Figure IV.6. This unit had a spontaneous rate of 4 spikes/sec and responded to tactile stimulation of the bilateral mouth with a short burst of activity followed by approximately 500 msec of suppression. However, this unit

Figure IV.5. Taste responses of a bimodal unit from the caudal portion of nucleus iobobulbaris (Fig. IV.2C) following application of amino acids and nucleotides to the ipsilateral maxillary barbel. This unit also responded to tactile stimulation of the ipsilateral mouth and oropharyngeal cavity (not shown). (AA - amino acid search mixture at the log molar concentration of each component, Con - well-water control, D-Ala - D-alanine, L-Ala - L-alanine, L-Arg - L-arginine, L-Pro - L-proline, Nuc - mixture of nucleic acid derivatives at the log molar concentration of each component; arrowheads indicate pressure pulse associated with switching the flow valve from background to stimulus)


AA
L-Ala
L-Arg
L-Pro
D-Ala
Nuc
Con
-2
-3
-4
-5
-6
-7
10 sec

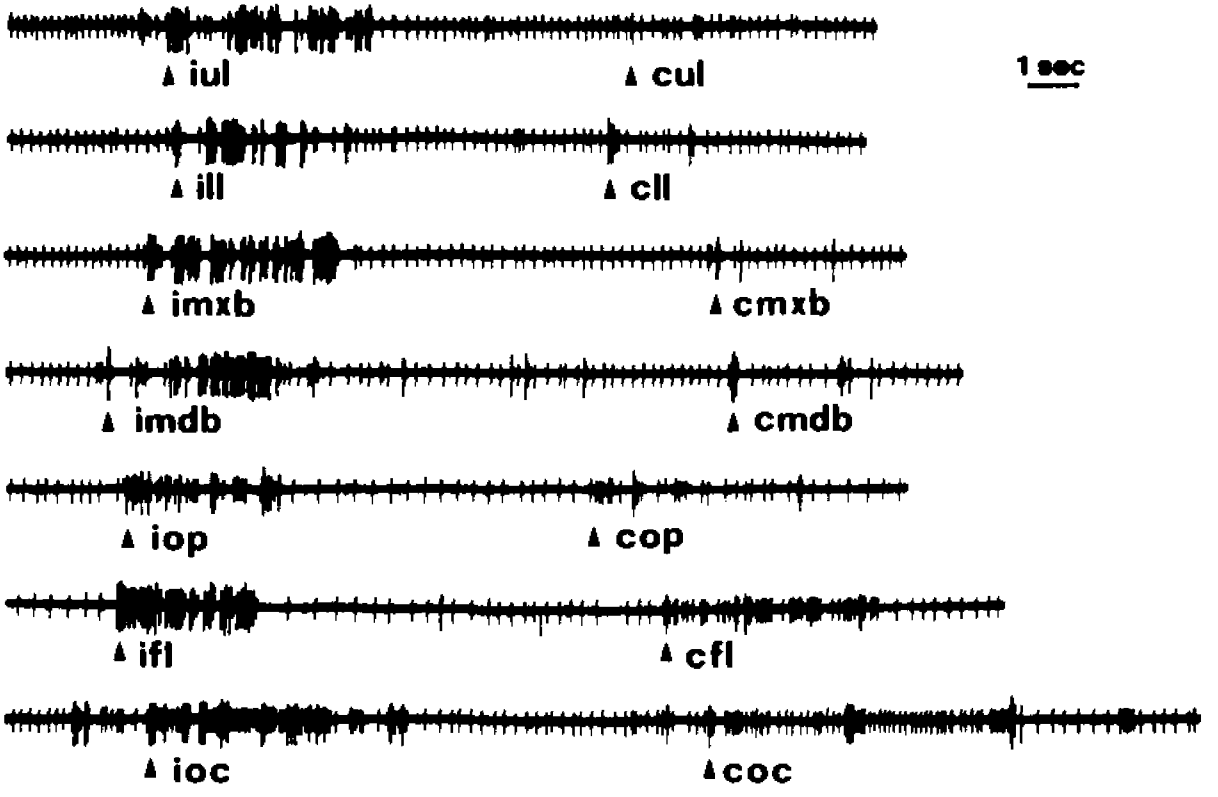


Figure IV.6. Tactile responses of two units from the rostrolateral nucleus lobobulbaris (Fig. IV.2D). The larger unit was facilitated by stimulation of the ipsilateral oropharyngeal cavity and extraoral body surface, while the smaller unit was facilitated by stimulation of the bilateral flanks and oropharyngeal cavity and responded to stimulation of the bilateral lips and barbels with short bursts of activity followed by brief suppression. (cfl - contralateral flank, cll - contralateral lower lip, cmdb - contralateral mandibular barbels, cmxb - contralateral maxillary barbel, coc - contralateral oropharyngeal cavity, cop - contralateral operculum, cul - contralateral upper lip, ifl - ipsilateral flank, ill - ipsilateral lower lip, imdb - ipsilateral mandibular barbels, imxb - ipsilateral maxillary barbel, ioc - ipsilateral oropharyngeal cavity, iop - ipsilateral operculum, iul - ipsilateral upper lip; arrowheads indicate onset of stimulation)

responded with sustained facilitation to stimulation of the bilateral flanks and oral cavity. Another unit from the same recording location (Fig. IV.2D) had little spontaneous activity and was facilitated by tactile stimulation of the ipsilateral oral and extraoral surfaces (larger unit in Fig. IV.6). Three units from the rostrolateral nucleus lobobulbaris also responded to taste stimulation with the amino acid mixture (each component at 10^{-3} M), but were not maintained long enough for complete testing with individual amino acids.

The third portion of the nucleus lobobulbaris consists of a parvicellular cell group (nLBp) associated with the dorsal region of the nucleus, adjacent to the tertiary gustatory tract. Thirteen single units were functionally isolated in this parvicellular portion and many displayed activity similar to units from the rostrolateral region (Table IV.1) Ten units had tactile RFs on both oral and extraoral surfaces, two were extraoral only, and one was only in the oropharyngeal cavity. Of the twelve units with extraoral RFs, six included the whole body, four were restricted to the head, and two responded only to stimulation of the mouth. Nine units from the parvicellular portion had bilateral receptive fields and four were ipsilateral only. All of the units recorded from this region were facilitated by tactile stimulation, but three had brief excitatory responses followed by suppression. Three units also responded to the amino acid search mixture, but were not further characterized.

Lateral thalamic nucleus - Six single units, all having spontaneous activity, were recorded from the lateral thalamic nucleus (nLT). Units from this nucleus typically had tactile RFs covering the

ipsilateral extraoral body surface (Table IV.1). This pattern of responsiveness was also prevalent in twelve multiunit recordings from the lateral thalamic nucleus (not shown). An example of a single unit isolated from the rostral portion of the lateral thalamic nucleus (Fig. IV.2E) with this response pattern is shown in Figure IV.7. Another unit from the rostral portion of this nucleus (Fig. IV.2F), however, differed significantly from the prevalent pattern (Fig. IV.8). This unit was facilitated by application of L-amino acids to the ipsilateral maxillary barbel, but was suppressed when D-alanine or the mixture of nucleic acid derivatives were applied to the same RF (Fig. IV.8A). Application of any of the tastants to the oropharyngeal cavity, including the L-amino acids, suppressed the activity of this unit (Fig. IV.8B). Tactile stimulation of both oral and extraoral surfaces also suppressed the spontaneous activity (Fig. IV.8C). This was the only bimodal unit isolated from the lateral thalamic nucleus and the only unit from this nucleus which responded with suppression (Table IV.1).

Nucleus subglomerulosus - The only unit isolated from the nucleus subglomerulosus responded to tactile stimulation of the entire ipsilateral extraoral body surface (Table IV.1). Five multiunit recordings were also identified in the nucleus subglomerulosus and their response patterns were identical to that of the single unit (not shown). All of the multiunit responses were tactile only, and none involved contralateral or oropharyngeal RFs.

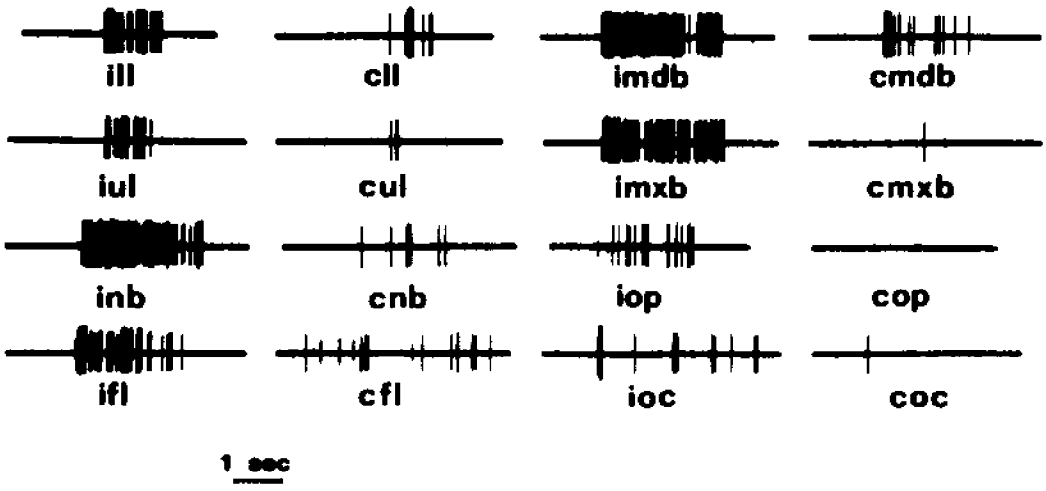
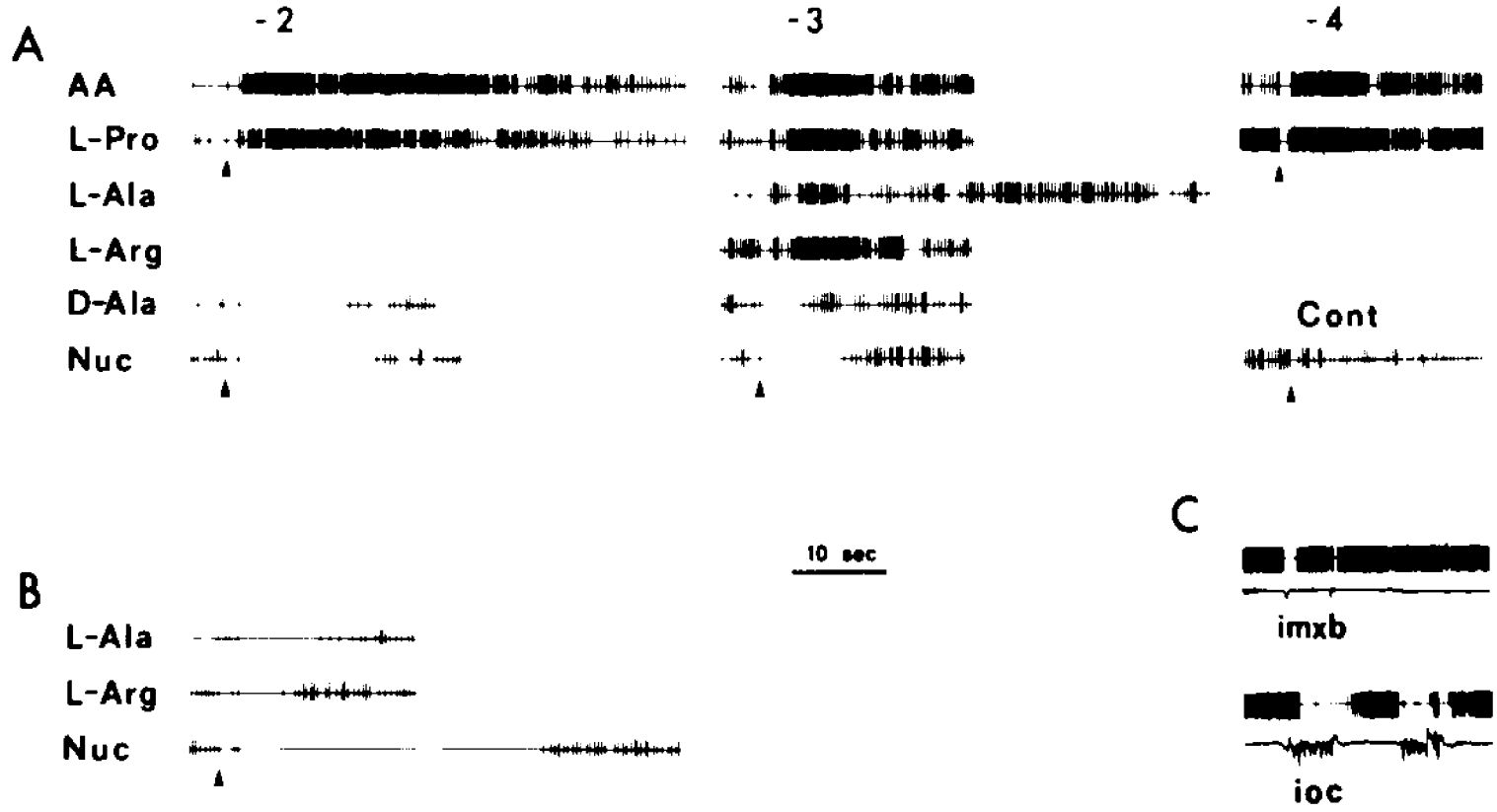


Figure IV.7. Tactile responses to stimulation of the ipsilateral extraoral body surface from a unit in the rostral region of the lateral thalamic nucleus (Fig. IV.2E). Slight responses were also produced by stimulation of the contralateral side. (cfl - contralateral flank, cll - contralateral lower lip, cmdb - contralateral mandibular barbels, cmxb - contralateral maxillary barbel, cnb - contralateral nasal barbel, coc - contralateral oropharyngeal cavity, cop - contralateral operculum, cul - contralateral upper lip, ifl - ipsilateral flank, ill - ipsilateral lower lip, imdb - ipsilateral mandibular barbels, imxb - ipsilateral maxillary barbels, inb - ipsilateral nasal barbel, ioc - ipsilateral oropharyngeal cavity, iop - ipsilateral operculum, iul - ipsilateral upper lip)

Figure IV.8. Bimodal responses from a unit in the rostral region of the lateral thalamic nucleus (Fig. IV.2F). A) Application of L-amino acids to the ipsilateral maxillary barbel facilitated the activity of this unit, while D-alanine and the mixture of nucleic acid derivatives suppressed it. B) Application of all tastants within the oropharyngeal cavity suppressed the activity of this unit. C) Tactile stimulation of the ipsilateral oropharyngeal cavity and extraoral surface also suppressed the unit. (AA - amino acid search mixture at concentration of each component, Cont - well-water control, D-Ala - D-alanine, imxb - ipsilateral maxillary barbel, ioc - ipsilateral oropharyngeal cavity, L-Ala - L-alanine, L-Arg - L-arginine, L-Pro - L-proline, Nuc - mixture of nucleic acid derivatives at concentration of each component; arrowheads indicate suppression of activity associated with switching water flow from background supply to test solution; numbers at top indicate exponents of molar concentrations of taste stimuli)


A
-2
-3
-4
AA
L-Pro
L-Ala
L-Arg
D-Ala
Nuc
Cont
B
10 sec
L-Ala
L-Arg
Nuc
C
imxb
ioc

DISCUSSION

The present study is the first to identify taste responsiveness from specified nuclei in the caudal inferior lobe of the channel catfish. Taste responses from this region of the diencephalon were found previously in catfish (Kanwal et al., '88) and goldfish (Demski, '81), but the specific nuclei in which the taste activity was found were not identified in either study. The present report provides physiological confirmation that several nuclei identified in previous anatomical studies as recipients of projections from the primary (Finger, '78; Morita and Finger, '85; Kanwal et al., '88; Chapter III) and secondary (Finger, '83; Lamb et al., '87; Kanwal et al., '88; Chapter III) gustatory nuclei relay gustatory information through the diencephalon of catfish. Neurons in the nucleus lobobulbaris (Figs. IV.4, IV.5) and lateral thalamic nucleus (Fig. IV.8) of the posterior tuberculum, and in the hypothalamic nucleus centralis (Fig. IV.3), responded to amino acids and nucleic acids applied to the oropharyngeal and extraoral epithelia.

Of the diencephalic nuclei receiving projections from the hindbrain gustatory centers (Chapter III), only the nucleus diffusus and nucleus subglomerulosus failed to display taste activity in the present study (Table IV.1). However, the techniques used for locating and recording single units made identification of responses particularly difficult in both of these nuclei. The nucleus diffusus consists of numerous, closely packed small cells and thus only multiunit responses were detected in this nucleus. Only three or four

electrode penetrations passed through the nucleus subglomerulosus, a relatively small nucleus located at the rostral margin of the gustatory projections into the inferior lobe. To detect gustatory responsiveness in these nuclei, a more focused approach is needed to bypass the larger cells of the nucleus lobobulbaris and nucleus centralis and specifically search for units in the lateral and rostral portions of the caudal inferior lobe.

Amino acids are potent gustatory stimuli for both the facial (Caprio, '75, '78; Davenport and Caprio, '82; Kanwal et al., '87; Wegert et al., '91) and glossopharyngeal/vagal (Kanwal and Caprio, '83) taste systems of the channel catfish. Similar to the previous reports of the responsiveness of peripheral taste fibers, facial lobe neurons (Marui and Caprio, '82), and neurons in the superior secondary gustatory nucleus (Chapter II), gustatory neurons in the inferior lobe of the channel catfish (present study) responded to several amino acids, but with greater responsiveness to L-arginine and L-alanine. Recent studies of peripheral facial nerves (Michel et al., '87; Kohbara et al., '90) and single neurons within the superior secondary gustatory nucleus (Chapter II) of catfish also identified nucleotides as a potent class of taste stimulants. The present study identified neurons within the nucleus centralis (Fig. IV.3), nucleus lobobulbaris (Figs. IV.4, IV.5), and lateral thalamic nucleus (Fig. IV.8) that responded to nucleotides. Furthermore, several units in the inferior lobe (see Figs. IV.4, IV.5, IV.8) responded in a dose-dependent fashion to both amino acids and nucleotides. These results indicate a

gustatory role for the nuclei in the caudal inferior lobe of the channel catfish; however, more comprehensive analyses of the chemically responsive neurons in the primary and secondary gustatory nuclei, as well as those in the currently described diencephalic nuclei, are necessary to understand the processing of gustatory information in catfish.

As in previous electrophysiological studies of gustatory nuclei in ictalurid catfishes (Biedenbach, '73; Marui and Caprio, '82; Kanwal and Caprio, '88; Kanwal et al., '88; Hayama and Caprio, '89; Chapter II) and the carp, Cyprinus carpio (Marui, '77, '81; Marui and Funakoshi, '79), tactile responses were more easily obtained from inferior lobe units than were taste responses. This tactile responsiveness revealed characteristic RF patterns between different gustatory nuclei in the caudal inferior lobe (see Table IV.1). Some of the differences in responsiveness between nuclei might provide clues concerning their respective roles in the processing of gustatory information.

The three regions of the nucleus lobobulbaris, which are distinguished by efferent projections and cell morphology (Chapter III), also display differences in resting activity and responses to tactile stimulation (Table IV.1). Units from the caudal magnocellular portion had lower spontaneous rates of activity (mean=1.65 spikes/sec, 60% with no spontaneous activity), and all of the sampled units responded to tactile stimulation with phasic facilitative bursts of activity (see Fig. IV.4). The receptive fields of these units typically included the entire ipsilateral extraoral body surface.

Projections of neurons within the caudal portion of the nucleus lobobulbaris of catfish terminate in the facial lobe of the medulla (Finger, '78; Morita and Finger, '85; Lamb et al., '87; Chapter III). The facial lobe receives peripheral input from facial nerves innervating extraoral taste buds (Herrick, '01; Finger, '76) and is essential for food identification and selection (Atema, '71). Neurons in the facial lobe respond to taste and tactile stimulation of restricted, topographically arranged receptive fields of the extraoral body surface (Marui and Caprio, '82; Hayama and Caprio, '89). The responsiveness of neurons in the nucleus lobobulbaris suggests that this nucleus might enhance incoming gustatory information in the facial lobe by providing a feedback loop for convergent facial information.

Units from both the rostrolateral magnocellular portion and the parvicellular portion had higher rates of spontaneous activity (means of 3.57 and 4.46 spikes/sec, respectively) than did units from the caudal magnocellular portion, and in both regions units displayed complex response patterns. Some units in the parvicellular portion of the nucleus lobobulbaris (23%) responded to tactile stimulation with a phasic increase in activity followed by a brief suppression, while more than half of the rostrolateral units responded with either the previously described pattern (23%) or with maintained suppression (38%). Common RF patterns for units from these two regions included a predominant responsiveness to taste and tactile stimulation of both oropharyngeal and extraoral surfaces, and units displayed a larger

percentage of bilateral responses than was found in the caudal lobobulbar cell group.

The rostrolateral magnocellular cells of the nucleus lobobulbaris project to the vagal lobe (Morita and Finger, '85; Lamb et al., '87; Chapter III), which is responsible for ingestive behavior (Atema, '71). Neurons in the vagal lobe have viscerotopically arranged RFs within the oropharyngeal cavity (Kanwal and Caprio, '88). Most of the units sampled from the rostrolateral lobobulbar cell group (85%) responded to both oral and extraoral stimulation. In addition, there was more suppression detected in this nucleus than was reported for any of the other gustatory nuclei in the channel catfish (Kanwal and Caprio, '88; Hayama and Caprio, '90; Chapter II; present study). These results indicate that the rostrolateral nucleus lobobulbaris might affect ingestion through a complex processing of facial and vagal neural information, since the response patterns of these lobobulbar cells are so different from their presumed target neurons. However, it is possible that the rostrolateral lobobulbar cells with large extraoral RFs do not project to the vagal lobe. Simultaneous intracellular recording and labeling in this cell group would provide definitive answers concerning the specific targets for neurons with different response patterns.

The parvicellular portion of nucleus lobobulbaris projects to the nucleus centralis pars dorsalis of the telencephalon (Kanwal et al., '88; Chapter III). The results of the present study indicate that these parvicellular lobobulbar neurons relay integrated facial and vagal gustatory information to the forebrain, but an understanding

of the role this nucleus plays in gustation awaits a more definitive identification of its connections.

The RF patterns identified in the present study also raise intriguing questions concerning the different projections of tertiary gustatory fibers from the superior secondary gustatory nucleus to the caudal inferior lobe (Kanwal et al., '88; Chapter III). Cell groups in the rostrolateral projection of tertiary gustatory fibers, the rostrolateral portions of nucleus lobobulbaris and nucleus diffusus, displayed both oral and extraoral RFs, while those nuclei in the caudal projection from the secondary gustatory nucleus, nucleus centralis and the caudal portion of nucleus lobobulbaris, had primarily extraoral receptive fields (Table IV.1). This distinction might represent a segregation within the secondary gustatory nucleus, or it could result from intrinsic processing within the inferior lobe. Further investigation of response patterns and projections of individual tertiary gustatory cells could determine whether such a segregation exists within the secondary gustatory nucleus.

Beyond establishing the involvement of the caudal portion of the inferior lobe in gustation, the present study leaves many questions unanswered regarding the functional role of each of the respective gustatory nuclei in this diencephalic region. Earlier behavioral studies using chronic electrical stimulation (Demski and Knigge, '71; Demski, '73; Savage and Roberts, '75) and lesions (Roberts and Savage, '78) in the caudal inferior lobe of different teleosts identified the importance of neural activity in this region for feeding behavior;

however, the specific mechanisms through which this activity affects feeding behavior remain unknown.

In the mammalian diencephalon, the lateral hypothalamus has long been associated with the elicitation of feeding behavior (Coons et al., '65). Neurons in the lateral hypothalamus receive ascending gustatory projections (Norgren, '76) and are responsive to taste stimulation (Norgren, '70). Possible mechanisms for the involvement of the lateral hypothalamus in feeding behavior are related to the descending projections from this nucleus to the nucleus of the solitary tract (van der Kooy et al., '84) and to the parabrachial nucleus (Saper et al., '79; Fulwiler and Saper, '84). Electrical stimulation in the lateral hypothalamus selectively facilitates the activity of gustatory neurons and suppresses the activity of thermal and tactile neurons in the nucleus of the solitary tract (Matsuo et al., '84). Lateral hypothalamic electrical stimulation also increases salivary secretion through the facilitation of preganglionic parasympathetic fibers (Matsuo and Kusano, '84). Feeding behaviors elicited by lateral hypothalamic stimulation were also associated with increased metabolism in the pathway from the lateral hypothalamus to the parabrachial nucleus (Roberts, '80). This hypothalamo-parabrachial projection was implicated in the reward effects of intracranial self-stimulation (Ferssiwi, et al., '87), a phenomenon which has been associated with the central processing of gustatory information (Olds, '62; Ganchrow et al., '81). These results indicate that the descending projections of the lateral hypothalamus in mammals are involved in a variety of feeding-related behaviors.

Similar to the mammalian lateral hypothalamus, the caudal inferior lobe of the channel catfish receives ascending gustatory input and projects back to both primary and secondary gustatory nuclei (see Chapter III). Unfortunately, nothing is known regarding the functional relationships between the gustatory nuclei in the inferior lobe and the primary and secondary gustatory nuclei. Future research should include lesioning or stimulating the magnocellular portions of the nucleus lobobulbaris or the lateral thalamic nucleus while recording the resultant changes in responses of neurons in the medullary gustatory lobes and the superior secondary nucleus to taste and tactile stimulation. In addition, further behavioral studies involving restricted lesions in the different nuclei in the caudal inferior lobe shown to be gustatory in the present study might provide a correlation between activity in a given nucleus and certain components of the feeding behavior of catfish.

Chapter V

Summary

Gustatory input from different regions of the extraoral and oropharyngeal surfaces of the channel catfish terminate in topographically organized locations within the facial and vagal lobes of the medulla, respectively. To investigate the processing of gustatory information that ascends from these medullary centers to higher brain regions, the response characteristics of neurons in each nucleus involved in the gustatory pathways must be determined and compared between nuclei. In this dissertation, the specific connections between the medullary gustatory centers and the next two levels of the ascending gustatory pathways, the superior secondary gustatory nucleus (nGS) and the posterior portion of the inferior lobe, were determined in the channel catfish and the responsiveness of neurons within the identified gustatory nuclei were analyzed electrophysiologically.

Information from different portions of the facial and vagal lobes converge onto neurons within the nGS. Neurons in the nGS responded to taste and tactile stimulation of large peripheral receptive fields (RFs), often including the epithelia of the oropharyngeal cavity and whole extraoral body surface. Taste responses to amino acids and nucleotides were dose-dependent, with detected thresholds from micromolar to millimolar concentrations. The organization of RF patterns within nGS did not display the exquisite topographical arrangement present in the medullary gustatory nuclei.

Anatomical labeling of secondary gustatory fibers (2G) from the medulla to the nGS supported the convergence identified electrophysiologically. The distribution of fibers from different

portions of both the facial (FL) and vagal (VL) lobes overlapped within the nGS. These results indicate that the nGS receives gustatory information from the medulla, but that the organization of that information changes dramatically from the highly organized topographic representations evident in both the facial and vagal lobes to a convergent representation of large portions of the body surface in the nGS.

Efferents from the nGS ascend in the tertiary gustatory tract (3G) to the caudal inferior lobe, where they terminate caudally in the nucleus lobobulbaris (nLB) and nucleus centralis (nCLI), and rostrolaterally in the nucleus diffusus (nDLI). Secondary projections from the FL also terminate in the nLB and in the nucleus subglomerulosus. The nLB forms three cell groups (caudal - nLB, rostrolateral - rl nLB, parvicellular - nLBp) which project to the FL, VL, and telencephalon, respectively. Cells from the nCLI project throughout the caudal inferior lobe and to the acousticolateral torus semicircularis and telencephalon, while the nDLI and nSG have intrinsic connections within the inferior lobe. The lateral thalamic nucleus (nLT) projects from this region back to the nGS. Through these identified connections several mechanisms for the processing of gustatory information can be proposed. Through the nLB, the rl nLB, and the nLT, information can feed back to the facial lobe, the vagal lobe, and the nGS, respectively, to affect the neural activity within those nuclei and to modulate medullary motor circuits. Projections from the nCLI to the torus semicircularis suggest an integration of

gustatory information with other sensory input, notably audition and lateral line mechanoreception and electroreception. The connections with the telencephalon allow for the involvement of gustation in learning processes and other complex behaviors.

Taste responses similar to those from nGS units were recorded from units in the nCLI, nLB, rl nLB, nLBp, and nLT, supporting the proposed gustatory role for these nuclei. Tactile responsiveness was distinct between different nuclei in the caudal inferior lobe. Units from the nCLI and nLB had lower spontaneous activity than those from other nuclei and typically had RFs including the whole extraoral body surface, ipsilaterally. Units from the rl nLB and nLBp had RFs often including both oral and extraoral surfaces, bilaterally, but rl nLB RFs typically included the whole body while nLBp RFs were often restricted to the head or mouth. To characterize fully the processing of gustatory information in these diencephalic nuclei, further investigation of the responsiveness within each nucleus is required; however, the apparent electrophysiological distinction between nuclei and their differential patterns of connections suggest that the gustatory nuclei in the inferior lobe of the channel catfish are involved in various different processing mechanisms. The elucidation of these mechanisms could provide critical information concerning not only the gustatory system of teleosts, but also the processing of sensory information in a variety of vertebrates.

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Appendix A

Abbreviations for Figures and Tables

Anatomical Abbreviations

2G - secondary gustatory tract
3G - tertiary gustatory tract
af - internal arcuate fibers of the mesencephalon
CM - corpus mammillare
comm - commissure of superior secondary gustatory nucleus
Dc - area dorsalis pars centralis of the telencephalon
FL - facial lobe
LL - lateral lemniscus
ltfLI - longitudinal terminal fields of the inferior lobe
nCLI - nucleus centralis of the inferior lobe
nDLI - nucleus diffusus of the inferior lobe
nGS - superior secondary gustatory nucleus
nLB - nucleus lobobulbaris
nLBp (or p) - nucleus lobobulbaris pars parvicellularis
nLT - lateral thalamic nucleus
nPGc - commissural preglomerular nucleus
nPGm - medial preglomerular nucleus
nRL - nucleus of the lateral recess
nSG - nucleus subglomerulosus
rl nLB - rostrolateral portion of nucleus lobobulbaris
Tel - telencephalon
tLB - lobobulbar tract
TS - torus semicircularis
V - fourth ventricle
VL - vagal lobe

Electrophysiological Abbreviations

AA (or AA mix) - amino acid search mixture
Adn - adenine
Ads - adenosine
ATP - adenosine triphosphate
cbr - contralateral branchial arches
cfl - contralateral flank
cll - contralateral lower lip
cmdb - contralateral mandibular barbels
cmxb - contralateral maxillary barbel
cnb - contralateral nasal barbel
coc - contralateral oropharyngeal cavity
Con (or Cont) - well-water control
cop - contralateral operculum
cul - contralateral upper lip
D-Ala - D-alanine
eso - esophagus
Gly - glycine
ibr - ipsilateral branchial arches
ifl - ipsilateral flank
ihd - ipsilateral head
imdb - ipsilateral mandibular barbels
imxb - ipsilateral maxillary barbel
inb - ipsilateral nasal barbel
ioc - ipsilateral oropharyngeal cavity
iop - ipsilateral operculum
iul - ipsilateral upper lip
L-Ala - L-alanine
L-Arg - L-arginine
L-Pro - L-proline
Nuc (or Nuc mix) - mixture of nucleic acid derivatives
pal - palate
Sp (or Sp Act) - spontaneous activity
Tact - tactile stimulation

Appendix B

Curriculum Vitae

Name: Charles Franklin Lamb IV

Born: May 12, 1958; Arcadia, CA

Education:

Edison High School (Huntington Beach, CA), June 1975.
Saddleback Community College (Mission Viejo, CA)
- Assoc. Arts degree (Biology), June 1979.
Humboldt State University (Arcata, CA)
- B. A. degree (Zoology and Fisheries), June 1983.
Louisiana State University (Baton Rouge, LA)
- M. S. degree (Physiology), August 1986.

Teaching:

Laboratory instructor - General Biology, General Zoology, Comparative Anatomy, Developmental Biology, Mammalian Physiology, and Sensory Neurophysiology.

Laboratory coordinator - Comp. Anat., Devel. Biol., Mamm. Physiol., and Sens. Neurophysiol.

Invited lecturer - General Zoology, Seminar on Science Education.

Techniques:

Electrophysiology - peripheral recording (hook electrodes, suction electrodes, electro-olfactogram), central recording (glass microelectrodes, tungsten electrodes), chronic microelectrode stimulation, spike analysis (amplitude analyzer, window discriminator, computerized unit analysis).

Anatomy - central and peripheral dissections, fixation, sectioning (freezing microtome, ultra-microtome), HRP and cobalt-lysine labeling, 3-dimensional computerized reconstruction.

Photography - light microscope, scanning electron microscope, transmission electron microscope, darkroom techniques.

Service:

President, Zoology and Physiology Graduate Student Organization (1988-1989, 1989-1990).
Graduate student representative on the Department of Zoology and Physiology Graduate Committee (1989-1990).
Member of the Student Advisory Committee for the Dean of the College of Basic Sciences (1990-1991).

Grants and Awards:

ZPGSO student travel award; April, 1988 ($100.00).

Sigma Xi Research Society, Grant-in-aid of Research; June, 1988 ($300.00).

ORF travel funds; December, 1988 ($100.00).

ISOT X student travel award; April, 1989 ($325.00).

LSU Alumni Association, Teaching Assistant of the Year; June, 1990 ($250.00).

Publications:

Lamb, C.F. IV. (1986) Tactile and Taste Responses in the Superior Secondary Gustatory Nucleus of the Catfish. M.S. Thesis, Louisiana State University.

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Vita

Charles Franklin Lamb IV was born in Arcadia, California, on 12 May 1958. He was the first child of Charles Franklin III and Barbara Lamb. He received his high school diploma from Edison High School (Huntington Beach, CA) in June, 1975. His undergraduate degrees include the Associate of Arts degree from Saddleback Community College (Mission Viejo, CA) in June, 1979, and the Bachelor of Arts degree in Zoology and Fisheries from Humboldt State University (Arcata, CA) in June, 1983. In August, 1983, he came to LSU to study under Dr. John Caprio in the Department of Zoology and Physiology. He received the Master of Science degree in August, 1986, for his thesis entitled "Taste and Tactile Responses in the Superior Secondary Gustatory Nucleus of the Catfish." He then married Judy Mae Banthin and moved with her to Kagoshima, Japan, where he worked as a research associate for one year with Dr. Takayuki Marui at the University of Kagoshima Dental School. In August, 1987, he returned to LSU to pursue his doctoral studies in the lab of Dr. Caprio. While completing his dissertation research he also visited the lab of Dr. Leo Demski at the University of Kentucky to study the role of the central nervous system of teleosts in behavior and the lab of Dr. Sven Ebbesson at the University of Alaska Marine Station at Seward to study neuroanatomical techniques. After the completion of his degree requirements at LSU, Charles will begin postdoctoral studies on the anatomy and physiology of the central nervous system of various vertebrates with Dr. Tom Finger at the University of Colorado Medical Center in Denver.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Charles Franklin Lamb IV

Major Field: Physiology

Title of Dissertation: Functional Morphology of Gustatory Centers in the Brain of the Channel Catfish, Ictalurus punctatus.

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

13 September 1991